STUDIES ON THE REPRODUCTIVE CYCLE

IN THE DOMESTIC FOWL

WITH PARTICULAR REFERENCE TO

THE INCIDENCE AND NATURE OF CERTAIN COMMON DEFECTS

IN EGGS

by

GANPATI PARSHAD SHARMA, M. Sc., Ph. D. (Punjab),
Institute of Animal Genetics, Edinburgh.

---- o O o ----

Submitted to the University of Edinburgh as a Thesis for the Degree of Ph. D., 1947.

---- o O o ----

TABLE OF CONTENTS

PART/-

PART II

PART /-

PART III

PART IV.

General Introduction:

There are two commonly met with types of defective eggs laid by the domestic hen that result in considerable financial loss to the poultry farmer and are frequent enough in their occurrence to affect perceptibly the potential food supply derived from the poultry industry.

The first of these results from the inclusion of blood or meat spots in eggs, and the second is the production of eggs so small as to make the majority of them unsaleable - the so-called "Miniature" eggs.

The presence of blood on the yolk of an egg was recorded by Aristotle as early as 300 B. C. and he attributed it to premature ovulation. Later, about 1600 A. D. Fabricius (Adelmann, 1942) during a demonstration of "sanguineous" eggs to his anatomy class described a liver-coloured body included in an egg. The occurrence of blood spots in eggs led both these authors to propound the theory that the yolk was directly formed from blood.

Miniature/-

Miniature eggs have been noted from time immemorial and various superstitions have been associated with them, the commonest of these being that such eggs were produced by old cocks and consequently they were named "Cock Eggs". It was believed that the "Cock Egg", if incubated, would hatch into a basilisk whose very look or breath would blast. Miniature eggs were also considered variously as good or evil omens. As "Witch Eggs" they were used to bring misfortune to an enemy, as "Luck Eggs" when thrown over a building any wish made while the egg was still in the air was certain of fulfilment.

Although both the defects have received considerable attention from scientific workers, it was thought that new light might be thrown on the nature of these abnormalities by consideration of their appearance and variation in eggs laid by birds of the flock of Brown Leghorns maintained at the Institute of Animal Genetics.

This stock, developed for genetical investigations on egg production and other economic qualities, has been derived from a few foundation birds, and no fresh blood has been introduced to it since/-

since 1931. The birds are kept intensively with no access to ground, and apart from the exigencies imposed by the war period the flock has been maintained on a fixed diet since its inception.

A number of inbred lines, selected and bred for particular characteristics have been carried for many years and constant observation and recording has provided a large body of reliable scientific material from which these investigations take their source.

Part I.

Variation in the Distribution of the Abnormalities.

The aim of this phase of the investigation was to determine the variations which occurred in the incidence (A) of blood and meat spots, and (B) of miniature eggs.

Section A: The Incidence of Blood and Meat Spots.

Introduction:

Although it has long been the opinion of poultry keepers that blood and meat spots are a consequence of a high rate of egg production Halnan and/-

and Day (1935) from their analysis of defects in 164,831 eggs laid at the West Suffolk egg-laying trials during 1931-34 found no correlation between the productivity of the bird and the incidence of faults. They showed also that the occurrence of a particular type of defect was an inherent characteristic, not only of the individual but of certain strains and was not primarily affected by diet or management of the stock. Defective eggs exhibited a tendency to increase during the summer but the cause of this phenomenon could not be established.

Van Wagenen, Hall and Wilgus (1937) opened 3,043 eggs laid by Single Comb White Leghorns and three American breeds, (Barred and White Plymouth Rocks, Single Comb Rhode Island Reds, and New Hampshires) at the Central and Western New York egg-laying tests and found that 23.8% contained meat spots and 8.4% blood spots. The eggs from the American breeds showed a greater incidence of meat spots than those from the Single Comb White Leghorn. No significant difference, however, was noted among the various breeds in the formation of blood spots. Their observations on eggs from the Single Comb White Leghorn in these particular laying/-

laying tests were substantiated by a further analysis by them of over 5000 eggs from the Cornell Experiment Station flock of Single Comb White Leghorns.

Quinn and Godfrey (1940) broke open about 6,000 eggs from Rhode Island Red , White Leghorn, White Wyandotte and crossbred pullets of these breeds and found no significant correlation between percentage of blood spots and egg production, egg weight or body weight. Their statistical analysis, however, showed significant breed and family differences with regard to spots both on the yolk and in the albumen. The mean percentage of total blood spots in the eggs of Rhode Island Red, White Wyandotte, White Leghorn, FI (Rhode Island Red x White Wyandotte) and FI (White Wyandotte x White Leghorn) was 62, 32, 4, 43 and 16 per cent respectively.

Lerner and Smith (1942) candled 152,570 eggs from a flock of Single Comb White Leghorns and found that the incidence of blood spots was 0.516 per cent. They noted significant seasonal differences as the incidence of blood spots was 0.401 per cent before and 0.651 per cent after April 1. Evidence was also presented by them showing the heritable nature of this defect.

Jeffrey/-

Jeffrey and Pino (1943) studied a ten-egg sample collected between 27th May and 29th June, 1942 from each of 238 Single Comb White Leghorn pullets which originated from 22 dams and 6 sires. On the basis of this study they concluded that the incidence of blood spots was primarily dependent on genetic factors. They also noticed that the environmental factor of confinement reduced the incidence of blood spots. They tried to increase the incidence of blood spots by frightening the birds at different hours of the day but without success.

Nalbandov and Card (1941 and 1944) also presented evidence to show that the tendency to lay blood spotted eggs was inherited. Contrary to the previous authors' findings range feeding, definitely helped in reducing the number and size of blood clots; confinement, on the other hand, had a reverse effect as the season advanced from December to July. In 60 per cent of the hens observed there was a decrease in the formation of blood clots with age while in 35 per cent this was reversed. The presence of these defects did not have any unfavourable effect on the hatching power of the eggs. Only White Leghorns were used/-

Nalbandov and Card (1944) that the meat spots originated as intrafollicular haemorrhages prior to ovulation. He also noticed marked individual differences between birds with respect to colour and size of meat spots. The incidence of red meat spots was highest during the first month of egg production but that of pale meat spots increased during the laying year. Both pale and red meat spots became smaller with the progress of the laying year. Seasonal changes did not have any marked effect on the incidence of white meat spots.

Lerner (1946) studied two series of Single Comb White Leghorn pullets hatched in consecutive years and found that the incidence of blood spots was in general higher in birds which survived their first laying year than in those which died.

Denton (1947) candled eggs from 85 Rhode Island Red hens during three periods (September-October, March-April and June-July) for the detection of blood and meat spots and subsequently opened to determine the candling errors. Of the eggs examined 72% contained meat spots and 3% blood spots.

Vitamin/-

Vitamin K, season and range feeding were found to have no appreciable effect on the incidence of the defects.

Material:

The data for the present investigation has been extracted from the records made on 292,548 eggs from the Institute flock during the six full laying years from 1940 to 1946. Detection of blood and meat spots of appreciable size is comparatively simply carried out by examination of the eggs over a source of light; the process is known as candling, and the apparatus in use at the Institute consists of a small rectangular box housing a 100 watt electric light bulb; on the upper side of the box is an ovoid aperture over which the eggs may be held and rotated to determine the normality or otherwise of their contents.

Since the data used in this section was obtained by this method no attempt will be made to distinguish between blood and meat spot eggs at present and for its purposes both will be included under the former designation. It is known also that

a/-

a percentage of these defects are too minute to be detected by this technique, so that the proportion identified would not be expected to be as great as in cases where every egg had been opened. Nevertheless, there seems no reason to assume that the method adopted here should markedly bias or distort any peculiarity of their distribution or trend in their variation though it might make them less evident.

Nine inbred lines and a mixed group of crosses are represented in the data; the birds range from one to six years of age but limitations of space necessitate discarding considerable numbers annually and the more advanced age groups are accordingly much reduced in relation to their initial population. For this analysis no selection of the birds was made on the basis of when they died or were discarded; on the other hand no discrimination against blood spots was adopted in deciding which birds should be retained in the flock. While the number of hens producing blood spot eggs may be slightly low on occasions (where birds have been disposed of early in the production year) the

proportion/-

proportion of defective to normal eggs should be fairly representative of the flock.

Results and Discussion:

The Frequency of Blood and Meat Spot Eggs.

The bulked figures for each age of bird in each production year are given in Table I. While the average proportion of defective eggs does not vary greatly from year to year, a tendency for older hens to produce them in greater numbers is evident. This will be further discussed when other sources of variation have been exposed. The average percentage of all eggs containing meat and blood spots (3.09 per 1000) is extremely low compared with that reported by most authors quoted, but is comparable with the figure for White Leghorns given by Lerner and Smith (1942). While a variation in this direction was to be expected on account of the use of candling for their detection, their higher incidence in the records of older birds should have weighted the figures in the opposite direction since previous work has mainly been confined to pullets.

Table I/-

Table I

The Frequency of Blood and Meat Spot Eggs in Hatching years and Age Groups.

1940-41.

Year Hatched	Total Egg Production	No. of Blood and Meat Spots	No. per 1000 Eggs
1940	27309	40	1.46

1941-42

1940	12708	58	4.56
1941	26646	97	3.64
TOTAL:	39354	155	3.94

1942-43

1940	8505	88	10.35
1941	15825	95	6.00
1942	20107	37	1.84
TOTAL:	44437	220	4.95

1943-44

1940	5983	83	13.87
1941	10627	35	3.29
1942	11316	20	1.77
1943	20957	19	0.91
TOTAL:	48883	157	3.21

1944/-

Table I (Continued)

1944-45

Year Hatched	Total Egg Production	No. of Blood and Meat Spots	No. per 1000 Eggs
1940	3438	48	13.96
1941	5353	64	11.96
1942	5705	11	1.93
1943	11327	11	0.97
1944	43406	34	0.78
TOTAL:	69229	168	2.43

1945-46

1940	1044	20	19.16
1941	1808	24	13.27
1942	2250	6	2.67
1943	2097	6	2.86
1944	17790	49	2.75
1945	38347	58	1.51
TOTAL:	63336	163	2.57
1940-46:	292548	903	3.09

Position/-

Position in Clutch:

Though previous workers had failed to substantiate the belief that blood spots are related to intensity of egg production and have been unable to find any connection between them and annual egg numbers, it was thought possible that their incidence might bear some relation to a particular part of the clutch. Their distribution on this basis has been set out in Table 2. Out of a total of 740 examined it will be seen that 378, or roughly half, were laid as one-egg clutches, i.e. no egg was laid on the preceding or succeeding day. Though the frequency of the various clutch sizes in the flock has not been determined the figures do not suggest an association between large clutch size and the presence of blood spots.

Table II/-

Table II

The Frequency Distribution of Clutch Position of Blood and Meat Spot Eggs for each size of Clutch.

No. of Eggs in the Clutch.	Position of Egg in the Clutch.									
	1st	2nd	3rd	4th	5th	6th	7th	9th	16th	TOTAL
1	378									378
2	123	75								198
3	41	29	18							88
4	13	12	6	8						39
5	4	4	5	2						15
6		1	1		1	1				4
7	1	1	1		1		1			5
9	1	1		1						3
11			2	2		1				5
13								1		1
15							1			1
18	1									1
19									1	1
27						1				1
TOTAL:	562	123	33	13	2	3	2	1	1	740

In the 2, 3, and 4-egg clutches the distribution of the data is indicative of a tendency for the incidence of these defects to decrease with later positions in the clutch. Tests made with chi-square confirm this view for both the 2-egg and 3-egg clutch groups yielded a highly significant value of the statistic (11.64 and 9.02 respectively). Though/-

Though with similar treatment the differences in the figures for the 4-egg clutches do not reach the level of significance, and the distribution in the larger runs does not at first glance show any particular trend, this is not unexpected with the smaller numbers of blood spots involved. Even in the latter however it may be noted that the proportion occurring in the first half of the cycles is greater than in the second (25:14). On the other hand it may be observed that a blood spot egg can occur at any position in the clutch, and this is particularly evident in the records of birds which produce many of these eggs.

Seasonal Variation.

Previous work on this aspect of the problem provides somewhat contradictory results for Jeffrey (1945) found blood spots more numerous at the beginning of production and least at the end (in August) while in Lerner and Smith's investigation (1942) the incidence was less prior to April than after it. Nalbandov and Card (1944) also found that in confined birds the production of blood clot eggs increased as the season advanced from December to/-

to July. It will be seen from Table 3a that the monthly distribution of defective eggs in the present material is not in agreement with Jeffrey's findings.

Table 3./-

Table 5

a. Monthly Incidence of Blood and Meat Spot Eggs from Pullets.

	Sept.	Oct.	Novr.	Decr.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.
Total Eggs	2146	5364	6885	9632	10587	14287	21429	22875	22222	19044	18150	12275
Blood Spots	3	2	4	15	24	29	45	31	45	25	20	15
do./1000	1.40	0.37	0.58	1.56	2.27	2.03	2.10	1.36	2.03	1.31	1.10	1.22

b. Test of Uniformity of Behaviour.

Period.	Unit	df	x^2	P	Differences.
Sept.-Aug.	1 mo.	11	26.180	$< 1\%$	Highly significant
Jan.-June	(1 mo.	5	8.389	ca.15%	Non-significant.
	(3 mo.	1	4.361	$< 5\%$	Significant
Sept.-Nov. v Dec.-Feb.	3 mo.	1	11.687	$< 0.1\%$	Highly significant.
March-May v. June-Aug.	3 mo.	1	6.753	$< 1\%$	Highly significant.

While the variation in the relative number of blood spots is not great, it does give the impression of following a curve which rises from October to the January-March season and then falls away again to the end of the laying year. A statistical comparison of all 12 months gives a chi-square value which is highly significant, indicating that real differences occur among the months (Table 3b), but the suggestion that the incidence of blood spots is falling off from January to June is not borne out when these six months are similarly compared among themselves for the probability that they are behaving alike is about 15%. When the data is grouped in 3-monthly classes however the difference between the two halves of this period does attain statistical significance but it is in the reverse direction to that obtained by Lerner and Smith (1942) for the incidence here is 0.21% in the first half and 0.16% in the second; it is obvious from the table even if the figure for the whole year were split in this way the difference would remain in the same direction.

When the first quarter of the production cycle is compared with the second and the third with the/-

the fourth, highly significant differences are exposed in both cases. If, therefore, there is barely sufficient evidence to confirm first impressions that the relative incidence of blood spots traces an annual curve at least it can be said that the incidence is higher in the middle of the cycle and lower in the initial and final months. This still allows of the theory that their occurrence is associated with rate of production for at the beginning and end of the laying year production is less intense than at other times.

Incidence in Inbred Lines.

Most of the authors quoted in the introduction to this section have noted hereditary tendencies in the incidence of blood spots, either on a basis of breed or family differences.

In the present study two birds (M 163 and O 221) were noticeably outstanding for the number of defective eggs they produced, and it seemed possible that an analysis of the data by breeding groups might expose differences among them.

Table 4/-

Table 4.

Variation by Lines and by Age.

A. Percentage of Blood and Meat Spots.

Group	1st Yr.	%	2nd Yr.	%	3rd Yr.	%	4th Yr.	%	5th Yr.	%	6th Yr.	%	Total
Dwarf													
Eggs:	8351		2240		1211		451		211				12464
Blood Spts.	3	0.04	2	0.09	0	0.0	0	0.0	1	0.47			6
Crossbreds													
	26572		11150		1978		1371						41071
	22	0.08	10	0.09	1	0.05	1	0.07					34
Intensity													
	20999		7188		3286		1957		905		339		34674
	16	0.08	7	0.10	9	0.27	1	0.05	1	0.11	0	0.0	34
Non-Moult													
	10810		4054		1825		812		187				17688
	10	0.09	4	0.10	5	0.27	3	0.37	1	0.53			23
Large Egg	12989		5440		2611		1318		573		105		23036
	19	0.15	11	0.20	6	0.23	4	0.30	0	0.0	0	0.0	40
Small Egg													
	8737		3874		1753		894		219				15477
	13	0.15	6	0.15	2	0.11	2	0.22	0	0.0			23

Breeding/-

Table 4A(Contd.)

Group	1st Yr.	%	2nd Yr.	%	3rd Yr.	%	4th Yr.	%	5th Yr.	%	6th Yr.	%	Total
Breeding	72619		27282		10161		4418		1707		453		116640
	120	0.17	73	0.27	18	0.18	12	0.27	3	0.18	2	0.44	228
Red	7869		2656		1396		625		316				12862
	14	0.18	25	0.94	8	0.57	2	0.32	1	0.32			50
Tumour	4968		3617		1599		1217		888		84		12373
	41	0.83	60	1.66	21	1.31	58	4.77	20	2.25	0	0.0	200
White	2858		1465		1114		523		240		63		6263
	27	0.94	35	2.39	70	6.28	70	13.38	45	18.75	18	28.57	265
Total:	176772		68966		26934		13586		5246		1044		292548
	285	0.16	233	0.34	140	0.52	153	1.13	72	1.37	20	1.92	903
do. excl. W.& T.	168946		63884		24221		11846		4118		897		273912
	217	0.13	138	0.22	49	0.20	25	0.21	7	0.17	2	0.22	438

Table 4 (Contd.)

B. The Percentage of Affected Birds.

Group	1st Yr.	%	2nd Yr.	%	3rd Yr.	%	4th Yr.	%	5th Yr.	%	6th Yr.	%
Dwarf												
Pop.	57		26		18		12		8		2	
Aff.birds	3	5.3	1	3.8	0	0	0	0	1	12.5	0	0
Crossbreds												
	155		94		18		14					
	19	12.3	8	8.5	1	5.6	1	7.1				
Intensity												
	118		43		21		14		6		4	
	14	11.9	6	14.0	9	42.9	1	7.1	1	16.7	0	0
Non-Moult												
	60		25		12		7		3			
	8	13.3	3	12.0	4	33.3	2	28.6	1	33.3		
Large Egg												
	117		48		28		18		8		1	
	18	15.4	10	20.8	4	14.3	4	22.2	0	0	0	0
Small Egg												
	74		35		20		15		5			
	12	16.2	5	14.3	2	10.0	2	13.3	0	0		
Breeding/												

Table 4 (B) Contd.

Group	1st Yr.	%	2nd Yr.	%	3rd Yr.	%	4th Yr.	%	5th Yr.	%	6th Yr.	%
Breeding	499		195		90		41		15		8	
	81	16.2	48	24.6	12	13.3	9	22.0	3	20.0	2	25.0
Red	56		20		14		9		6			
	12	21.4	13	65.0	4	28.6	1	11.1	1	16.7		
Tumour	36		28		13		11		9		2	
	15	41.7	17	60.7	8	61.5	6	54.5	5	55.6	0	0
White	22		16		15		14		8		4	
	9	40.9	9	56.3	8	53.3	6	42.9	2	25.0	1	25.0
Total	1194		530		249		155		68		21	
	191	16.0	120	22.6	52	20.9	32	20.6	14	20.6	3	14.3
do. excl. W & T.	1136		486		221		130		51		15	
	167	14.7	94	19.3	36	16.3	20	15.4	7	13.7	2	13.3

Table 4 (Contd.)

C. Test of Uniformity of Lines (Pullets only).

		df	x^2	P	Differences
% Blood Spots	All Groups	9	276.52	< 0.1%	Highly significant
	excl. W. & T.	7	25.26	< 0.1	" "
	excl.W.T.D. & Xb.	5	11.74	< 5	Significant
% Affected Birds	All Groups	9	37.40	< 0.1	Highly significant
	excl. W. & T.	7	8.76	ca 30	Non-significant

Table 4 (Contd.)

D. Test of Uniformity of Age Groups.

		df	x^2	P	Differences
% Blood Spots	All Groups	5	741.17	<0.1%	Highly significant
	Excl. W. & T.	5	27.79	<0.1	" "
	Excl.M 163 and O 221	5	73.31	<0.1	" "
	Excl. Pullets	4	246.76	<0.1	" "
	Excl.Pullets and M 163 and O 221	4	1.005	>90.0%	Non-significant
% Affected Birds	All Groups	5	12.78	ca 3%	Significant
	Excl. Pullets	4	1.20	ca 85%	Non-Significant

In Table 4, the data for blood spot eggs and birds producing them has been classified according to lines and to years of production. Nine inbred lines are represented and one group called "cross-breds", is made up of various crosses between the lines, mainly first crosses, but does not include any involving the "Dwarf", "White", or "Tumour" lines.

Examination of the material for the pullet year, where the population is at its maximum shows that the proportion of both blood spots and affected birds is low in the "Dwarf" group and very high in the "White" and "Tumour" groups. In the remaining lines and the cross-breds, their incidence is somewhat higher than in the Dwarf but the range is not great. Though the smaller populations in later production years make some of the data unreliable it seems clear that the "Dwarf", "White" and "Tumour" lines maintain their original position in relation to the other groups.

If the proportion of blood spot eggs from pullets is tested for uniformity in the various lines by the chi-square method, the probability of their homogeneity is extremely low, far less than 0.1%.

Exclusion/-

Exclusion of the "White" and "Tumour" lines reduces the value of chi-square considerably but P is still less than 0.1%. The further elimination of the "Dwarf" and "cross-bred" figures from the data again reduces the size of the statistic but P falls about 5%, indicating that real differences may exist even among the remaining lines. (Table 4c). Similar treatment of the figures for affected birds shows that significant differences among lines disappear when White and Tumour lines are excluded from the comparison. Comparison of the percentage of first year blood spots and affected pullets in Tables 4A and B, however, reveals that the order of their incidence in the remaining lines is the same so that the inability to detect significant differences among them in respect of affected birds may be due to the smallness of the populations, and not necessarily to variations in the number of defective eggs per bird.

Variation with Age.

In dealing with the effect of age on the incidence of blood spots, a difficulty arises in that the original population is annually reduced by culling and/-

and mortality. In culling the birds no discrimination was made against those producing blood spots so that unless the defect is associated with other undesirable qualities or with pathological conditions leading to premature deaths, the reduced samples available at succeeding stages should remain representative of the flock. On the other hand, if any such factors were operative, the proportion of blood spots might be expected to be higher in the earlier years.

In Table 4A where the influence of age is shown, the total figures from all lines actually indicate the reverse of this for there is a consistent rise in the percentage from the 1st to the 6th year of production. The proportion of birds affected (4B) is about 16% in the 1st, 14% in the 6th year, and just over 20% in the intervening ones so the increase in defective eggs cannot be related to a parallel rise in the number of birds producing them.

In the separate lines the phenomenon is not always detectable but it is very pronounced in the "White" line and, despite irregularities, in the "Tumour" one. When the data relative to these two groups is removed the incidence of blood spots becomes

much/-

much more uniform at the different ages and an upward trend is no longer evident, but the pullets retain their position with the lowest percentage.

Statistical treatment of the data confirmed these views (Table 4D); with or without either "White" and "Tumour" or the pullet eggs, the age groups showed highly significant differences among themselves, but when both these categories were excluded the remaining material showed no evidence whatever of heterogeneity. The two exceptional birds mentioned earlier (M 163 and O 221) belonged to the "White" and "Tumour" lines; in post-pullet years large series of their eggs were opened in a search for small blood spots and this might have unbalanced the data for age groups. It was, in fact, found that exclusion of their eggs had a similar effect to eliminating the lines to which they belonged so the apparent difference between the latter and the other lines in respect of age may be accidental due to the change in technique of identifying affected eggs from these two birds. (Their exclusion from the pullet year, where the data was used to examine difference between lines, does not affect that analysis for the blood spot/-

spot incidence still remained high in "White" and "Tumour" lines in comparison with the remaining ones). In regard to affected birds all significant differences between ages disappeared with the exclusion of the pullets from the comparison.

It must be concluded, therefore, that the incidence of blood spots is definitely lowest in pullet eggs, but a continuing rise with age cannot be established. Further investigation of the behaviour of the high incidence lines does not seem to be justified at present in view of the small populations and diminishing size of the age groups.

In connection with the conclusions just made attention may be drawn to Lerner's (1946) findings that, after adjustment for seasonal and hereditary differences, the incidence of blood spots tended to be higher in birds which survived the first laying year than in those which died. There appears to be a measure of similarity between the two sets of results though the post-pullet year figures here represent the effect of culling as well as mortality. On the evidence of his own material Lerner discarded the possible theory that higher egg production of the survivors/-

survivors might be responsible for the higher incidence of blood spots. It is interesting to note, however, that this factor would certainly operate in so far as culling the present stock was concerned, but, on the other hand, the two high incidence lines were among the least productive groups in the flock studied here.

Since a comparison of the variation in incidence of blood spots and of miniature eggs in the flock will be made at the end of this part of the thesis Table 5 has been appended: it shows the distribution of blood spots from affected birds which were retained or survived the same number of years and so constitute a constant population; also the numbers of these birds which were affected in the respective years.

Table 5/-

Table 5.

Data from Affected birds Surviving the Same Number of Years

Birds		No. of Blood Spots					No. of Birds Affected.				
Surviving	Pop.	1st	2nd	3rd	4th	5th Yr.	1st	2nd	3rd	4th	5th Yr.
5 years	30+	16	48	28	16	16	10	20	17	12	12
4 years	63	33	75	54	40		21	34	32	30	
3 years	110	71	104	76			44	56	50		

+ M 163 and O 221 excluded.

Arranged in this way the figures confirm the findings in the full data of a lower incidence of blood spots and of affected birds in the pullet year, but they also show up a further peculiarity - that the number of blood spots, the number of affected birds, and the number of blood spots per affected bird, all tend to be at a maximum in the second year. This is obviously another facet of the phenomenon described by Lerner (1946), but in this case the population remains constant so that the retention of birds in the higher production groups is again ruled out as an explanation.

Section B/-

Section B. The Incidence of Miniature Eggs

Introduction:

In a comprehensive paper, Pearl and Curtis (1916) have discussed the various aspects of the problem of the miniature or "dwarf" eggs and classified them according to shape as prolate-spheroidal or cylindrical, the latter being quite rare.

As early as 1898, Féré stated that the eggs in the beginning and at the end of a clutch are smaller than the intermediate ones. Somewhat similar views were expressed by Lewis (1913) and in an unsigned article on "Xenia in Fowls" in the Journal of Heredity (Vol. 6, 1915).

Pearl, Surface and Curtis (1911), however, state that although the laying of miniature eggs is popularly supposed to mark the end of a laying period, this belief is without foundation in fact and they may be produced at any time. Warner and Kirkpatrick (1916) have also pointed out that the small eggs are not necessarily laid at either end of the clutch. Such eggs, according to them, are more common during periods of heavy egg production.

Material/-

Material.

The data for the investigation has been extracted from the records made on 292,548 eggs produced by the Institute flock during the six full laying years from 1940 to 1946. Altogether 427 miniature eggs occurred; they varied in size from 0.89 grm. to 53.0 grm. Miniatures have been defined as eggs whose weights clearly fall below the general limits of variation in egg size for a particular bird, and they may vary from 1/50th, to $\frac{3}{4}$th of the characteristic weight.

Results and Discussion:

The Frequency of Miniature Egg Production and of Affected Birds.

Warner and Kirkpatrick (1916) have shown that during the 3rd and 4th egg laying contests at Storrs, Connecticut, out of 199,137 eggs laid, 103 weighed less than 0.09 lb. Thus, according to their figures, one egg in every 1933 was a miniature.

Pearl and Curtis (1916) calculated the percentage of miniature eggs for each of the two years/-

years of maximum miniature egg production in their flock (1911-12 and 1914-15) and found that one egg in every 1158 was a miniature.

Similarly Crew (1930) has stated that "If the eggs laid by a large number of fowls are examined it will be found that about one in every 2000 is thus abnormal".

In the present study the comparable figure for the total data is 1 in 685. Table 6 shows the incidence by production years and ages for the six years studied. For the pullets the number per 1000 varied from 0.65 in 1944-45 to 2.16 in 1945-46, the latter being almost double the next highest proportion of 1.13, occurring in 1941-42. While this suggests differences among the years, an analysis of this aspect has not been undertaken since changes in the size of population in the various lines from year to year might affect the results. There is some suggestion, however, of a rise in incidence in the higher age groups.

Table 6/-

Table 6

The Frequency of Miniature Eggs in Hatching Years and Age Groups.

1940-41

Year Hatched.	Total Egg Production.	No. of Miniature Eggs.	No. per 1000 Eggs.
1940	27309	25	0.92

1941-42

1940	12708	14	1.10
1941	26646	30	1.13
TOTAL	39354	44	1.12

1942-43

1940	8505	24	2.82
1941	15825	38	2.40
1942	20107	19	0.94
TOTAL	44437	81	1.82

1943-44

1940	5983	24	4.01
1941	10627	48	4.52
1942	11316	6	0.53
1943	20957	15	0.72
TOTAL	48883	93	1.90

1944/-

Table 6 (Continued)

1944-45

Year Hatched.	Total Egg Production.	No. of Miniature Eggs.	No. per 1000 Eggs.
1940	3438	20	5.82
1941	5353	20	3.74
1942	5705	1	0.18
1943	11327	8	0.71
1944	43406	28	0.65
TOTAL	69229	77	1.11

1945-46

1940	1044	4	3.83
1941	1808	3	1.66
1942	2250	4	1.78
1943	2097	3	1.43
1944	17790	10	0.56
1945	38347	83	2.16
TOTAL	63336	107	1.69
1940-46	292548	427	1.46

According/-

According to Warner and Kirkpatrick (1916), 85 birds out of a total of 1820 (4.67%) laid one or more miniature eggs during the laying contests already mentioned. Pearl and Curtis (1916) also found that about 5% of the birds in their flocks produced at least one miniature egg. The percentage of pullets in this flock laying miniature eggs during the years under review was 11.6% or approximately twice the figure recorded from American observations. Strict comparisons, however, are not possible since in this study the upper weight limit for miniatures falls within the size range of eggs produced by an average flock of hens. These, by definition, have been classified as miniatures because they are much below the characteristic egg weight for the individual bird.

Position in the Clutch.

Innumerable references to the belief that miniature eggs mark the beginning or end of a laying period can be found in the literature from an early period to the present day. It has already been noted, however, that Pearl, Surface and Curtis (1911) disagree with this and state that miniature eggs can be/-

be produced at any time. Similar views have been expressed again by Pearl and Curtis (1916) as well as by Warner and Kirkpatrick (1916) and Crew (1930).

Table 7/-

Table 7

Distribution of Miniature Eggs in relation to position in Clutch (1940-45).

No. of Eggs in the Clutch	Position of Egg in the Clutch										
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	Total
1	132										132
2	41	49									90
3	10	17	14								41
4	4	5	8	7							24
5	3	3	2	4	3						15
6			1	1		3					5
7				2		1					3
8					1						1
9				1					1		2
10	1										1
12		1							1		2
13						1					1
14							1	1		1	3
TOTAL	191	75	25	15	4	5	1	1	2	1	320

While the numbers of miniature eggs occurring in the second half of the clutch were slightly greater in the distribution observed here and presented in Table 7, it was not possible to expose any significant connection between frequency and position in the clutch.

Seasonal/-

Seasonal Variation.

According to Warner and Kirkpatrick (1916) it is during periods of heavy egg production that miniature eggs are more common. Similarly Pearl and Curtis (1916) noted that most of them were laid between March and July, but that the increase at this time was greater than would be expected on the basis of increased egg production alone; they further observed that dwarf egg production, unlike multiple-yolked egg production, is not associated with immaturity, and that "pullets are increasingly likely to lay dwarf eggs up to the time they are one year old and that the chances then decrease up to the end of the pullet year".

Table 8/-

Table 8.

Seasonal Variation in Incidence of Miniature Eggs in Pullets.

	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.
Number	3	7	14	6	14	14	25	26	28	14	17	21
No./1000	1.40	1.30	2.03	0.62	1.32	0.98	1.17	1.14	1.26	0.74	0.94	1.71
	← 1.67 →			← 0.99 →			← 1.19 →			← 1.05 →		
	For single months χ^2 = 15.14; df. 11; P = ca 19%; non-significant											
	Quarterly χ^2 = 4.65; df. 3; P = 20%; non-significant											

Table 8 shows the monthly numbers and the proportion of miniature to normal eggs for the pullet years. While the actual numbers rise as the laying cycle progresses, it is clear that the proportion does not vary in any consistent manner, and this is borne out by a chi-square test which yields a probability of homogeneity of about 19 per cent. Even when the figures are grouped in three-monthly periods, and suggest a higher incidence in the first quarter, the statistic indicates that the differences are no more significant.

This disagreement with Pearl and Curtis' findings necessitates a further examination of their work; they present the monthly number of miniatures for nine annual flocks over 8 years but only compared the distribution of miniatures and normal eggs in the two with the highest numbers of the former. Their conclusions depend on the high proportion of dwarf eggs in June and July but it is noticeable that in the 8 years data the percentage of the annual total of dwarf eggs is very variable in these months and in the two test years is particularly high. It is therefore doubtful/-

doubtful if their conclusions are really applicable to the whole of their material.

Incidence in Inbred Lines and Age Groups.

No guidance has been obtained from previous researches as to the variations to be expected in these categories: references to hereditary tendencies in miniature egg production have not been found, and Pearl and Curtis (1916) were unable to decide whether or not a bird was more inclined to lay a miniature egg in her second or third year than in her first.

Table 9/-

Table 9.

Variation by Lines and Age.

A. Percentage of Miniature Eggs.

Production Year:	1st		2nd		3rd		4th		5th		6th		TOTAL	
	No.of miniatures.	%	No.of miniatures.	%	No.of miniatures.	%	No.of miniatures.	%	No.of miniatures.	%	No.of miniatures.	%	No.of miniatures	%
Red	36	0.46	2	0.08	3	0.22	2	0.32	1	0.32	0		44	0.34
Dwarf	30	0.36	21	0.94	28	2.31	19	4.21	8	3.79	0		106	0.85
White	10	0.35	5	0.34	13	1.17	15	2.87	9	3.75	1	1.59	53	0.85
Intensity	30	0.14	5	0.07	6	0.18	4	0.20	0		0		45	0.13
Small Egg	11	0.13	3	0.08	2	0.11	0		0		0		16	0.10
Large Egg	10	0.08	10	0.18	9	0.34	0		0		0		29	0.13
Breeding	49	0.07	18	0.07	6	0.06	3	0.07	4	0.23	3	0.66	83	0.07
Tumour	3	0.06	7	0.19	2	0.13	0		1	0.11	0		13	0.11
Non-moult	5	0.05	0		3	0.16	0		0		0		8	0.05
Crosses	16	0.06	5	0.04	4	0.20	5	0.36	0		0		30	0.07
TOTAL	200	0.11	76	0.11	76	0.28	48	0.35	23	0.44	4	0.38	427	0.15

Table 9.

Variation by Lines and Age.

B. Percentage of Affected Birds.

Production Year:	1st		2nd		3rd		4th		5th		6th	
	No.of Affected Birds	%	No.of Affected Birds.	%	No.of Affected Birds.	%	No. of Affected Birds.	%	No.of Affected Birds.	%	No.of Affected Birds.	%
Red	12	21.4	2	10.0	1	7.1	2	22.2	1	16.7	0	
Dwarf	16	28.1	9	34.6	8	44.4	6	50.0	3	37.5	0	
White	3	13.6	3	18.8	8	53.3	6	42.9	2	25.0	1	25.0
Intensity	23	19.5	4	9.3	5	23.8	4	28.6	0		0	
Small Egg	11	14.9	3	8.6	2	10.0	0		0		0	
Large Egg	8	6.8	3	6.3	1	3.6	0		0		0	
Breeding	43	8.6	17	8.7	5	5.6	2	4.9	2	13.3	3	37.5
Tumour	3	8.3	6	21.4	2	15.4	0		1	11.1	0	
Non-Moult	5	8.3	0		1	8.3	0		0		0	
Crosses	14	9.0	4	4.3	1	5.6	4	28.6	0		0	
TOTAL:	138	11.6	51	9.6	34	13.7	24	15.5	9	13.2	4	19.0

Table 9.

C. Test of Uniformity of Incidence of Miniature Eggs.

		df	χ^2	P	Differences
In Lines	All Groups	9	170.26	< 0.1%	Highly significant
	Excl.R.& D.	7	41.26	< 0.1	" "
	Excl.R.D.W. & I.	5	5.39	ca 37.0	Not significant
In Age Groups	All groups	5	128.20	< 0.1	Highly significant
	Excl.D.I. & W.	5	14.01	< 2.0	Significant
	do. excl.5th & 6th yrs.	3	7.51	> 5.0	Barely significant

Table 9.

D. Test of Uniformity in Proportion of Affected Birds.

		df	x^2	P	Differences
In Lines	All Groups	9	37.40	<0.1%	Highly significant
	excl.R & D	7	16.60	2.0	Significant.
	excl.R.D.& I.	6	5.03	ca 55	Not significant
In Age Groups	All Groups	5	6.57	ca 25	Not significant

In Tables 9 A & B the numbers and percentages of miniature eggs and affected birds are classified according to lines and age. As the same body of material was used here as in the study of blood spots, the total eggs and population relative to each item in the tables have been omitted since they are already available in Tables 4 A & B.

Though from the first table it can be seen that the range of variation in the incidence of miniature eggs from pullets in the different lines is less than in the case of blood spots, the "Red", "Dwarf", and "White" lines form a group which appear to be more markedly affected while "Intensity" and "Small Egg" occupy an intermediate position in the range. In later production years "Dwarf" and "White" maintain their position and are the only lines in which the rising incidence with age suggested by the total figures can be clearly traced. That the third line in the group appeared to diverge in behaviour was due to the presence of a single pullet, not represented in later years, which produced 23 miniature eggs randomly spaced throughout the laying cycle. Though 25 of them, that is roughly a fifth of the affected/-

affected pullets, laid more than one miniature, no other bird has produced more than eight. Chi-square tests relative to these observations showed that even when "Red" and "Dwarf" were excluded highly significant differences were present among the groups of pullets (Table 9C) but that there were no grounds for assuming that the remaining lines were not behaving alike when "White" and "Intensity" were also eliminated for by doing this the probability of homogeneity was increased to 37%. Confirmation of highly significant differences among the six age groups was obtained with a chi-square value of 128 but attempts to show their dependence on the presence of particular lines were unsuccessful: the most obvious rises in incidence occurred in the "Dwarf", "White" and "Large Egg" lines but without them the probability of homogeneity was not raised above 2%, and when the last two years (which did not include all lines) were cut out it still remained in the region of 5%.

As regards the proportion of pullets producing/-

producing miniature eggs shown in Table 9 B, the differences between the lines appear more striking: "Red" and "Dwarf" again show the highest percentage with roughly a quarter of their population affected, and "Small Egg" are again intermediate in position, but "Intensity" and "White" have changed places in the series. However the latter was the smallest group, represented by only 22 pullets, and the figure of 13% must be regarded as but a rough estimate of the true incidence since the addition or absence of a single bird would be enough to place it in the higher or lower groups respectively. In this case the value of chi-square indicated marked differences among the lines which could only be reduced below the level of significance by exclusion of "Red", "Dwarf" and "Intensity" (Table 9 D).

Though increases in the proportion of affected birds with age in the various lines are suggested by the table, the small populations make them unsuitable for detailed analysis and the total figures/-

figures for all groups give a value of 6.57 for chi-square, with a probability of 25%, which is well within the limits of variation expected in a homogeneous population.

Tabulation of the data from affected birds surviving the same number of years has been carried out in Table 10: with one exception the number of miniature eggs rises with each production year. The number of birds affected in successive age groups also gives evidence of increasing and though this could not be proved statistically for the complete data, the trend here seems sufficiently consistent to suggest that it might prove significant with larger populations in the third and later years.

Table 10/-

Table 10.

Data for Affected Birds Surviving the Same Number of Years.

Birds		No. of Miniatures					No.of Affected Birds				
Surviving	Pop.	1st	2nd	3rd	4th	5th Yr.	1st	2nd	3rd	4th	5th Yr.
5 years	18	4	7	12	16	23	3	6	8	5	9
4 years	44	19	18	43	48		14	12	18	24	
3 years	69	32	40	76			22	23	34		

SUMMARY AND CONCLUSIONS

The Incidence of Blood (and Meat) Spots and of Miniature Eggs.

The material for the present study was extracted from the records made on 292,548 eggs from the Brown Leghorn flock at the Institute of Animal Genetics during six full laying years from 1940 to 1946. Blood spots were identified by candling; a total of 903 were encountered and there were 427 miniature eggs.

The proportion of blood spot to total production was 0.31% for the whole flock, and 0.16% for the pullets; 16% of the latter produced one or more blood spot eggs. The comparable figures for miniatures were 0.15% for the whole flock and 0.11% for pullets; 11.6% of pullets were affected.

In pullets the proportion of blood spots rose as the season progressed and then fell off again towards the end of the laying cycle. No similar seasonal trend could be identified in the incidence of miniatures.

More/-

More blood spots tended to occur in the first half of a clutch than in the second, but miniatures showed no association with any particular position in the clutch.

Among the various inbred lines differences were exposed in the proportion of both blood spots and miniatures occurring in them, and in the percentage of birds affected. The lines which showed a high incidence of the two abnormalities were not the same except in one case.

There was a rise in the percentage of blood spots after the pullet year, but in the absence of the two high incidence lines there was no significant difference among post-pullet years. Differences in the proportion of affected birds were not detectable among post-pullet age groups. In constant populations the number of blood spots and of affected birds appeared to be at a maximum in the second year.

The percentage of miniatures showed a general tendency to rise as the birds became older, and the differences among age groups did not seem to be/-

be restricted to particular lines. Variations in the proportion of birds producing miniatures at different ages were not significant but in constant populations the number of miniatures and affected birds both tended to increase with age.

While differences in the incidence of both abnormalities occur among the various inbred lines of the flock, and thus suggest that both have some hereditary basis, their behaviour diverges in other respects.

The tendency of miniature eggs, and of birds producing them, to become more prevalent with age points to a slight but progressive weakening in some physiological process, but the lack of any similar tendency for blood spots after the second year eliminates them as symptoms of senility.

On the other hand though the seasonal variation of the latter followed the trend of the annual production cycle, the evidence from the constant populations, and the fact that the high incidence lines were not particularly good layers, eliminates high/-

high production as such from the direct causal agencies. It may be, however, that blood spots are associated with a rate of production which is high for the particular bird concerned: while this would not account for the high second year incidence, it would be in line with the decreasing numbers obtained from more aged birds.

Part II

Morphology of The Abnormalities

Here the structural and histological features of (A) Blood and Meat Spots and (B) the constituent contents of miniature eggs have been examined in an effort to elucidate their nature and origin.

Section A: Morphology and Nature of Blood and Meat Spots.

Introduction:

Meat spots have been frequently described as pieces of glandular tissue or abnormal growths which have been torn from the wall of the oviduct during the passage of an egg through it. Benjamin and Pierce (1937) believe that these are due to an abnormal condition of the oviduct. Burmester and Card (1938), however, showed that red blood corpusles or a significant amount of iron was present in all except 3 per cent of the meat spots.

Evidence has been presented by Nalbandov and/-

and Card (1941 and 1944) to indicate that the haemorrhages which resulted in the formation of blood spots occurred before ovulation. These blood clots were, according to them, transformed into meat spots by changes in pH and high environmental temperatures. The transformation took place either before ovulation, or during egg formation, or even after the egg had been laid. They attempted to prevent intra-follicular haemorrhage experimentally by the administration of vitamins A. C. D. E. K. and P. but were unsuccessful.

Lucas (1946) prepared smears from blood spots and demonstrated macrophages and fibroblasts in addition to intra-vascular cell types. (It was also suggested that the transfer of viable parent connective tissue cells to the egg may have some significance in the problem of transmission of lymphomatosis and other avian diseases through the egg).

Denton (1947) observed that in general the eggs from the individual hen contained meat spots which were predominantly one colour. The transforma-

tion/-

tion of a blood clot into a meat spot, according to him, was influenced only by the time which elapsed between the haemorrhage and the subsequent removal of the blood clot from the body cavity along with the egg content.

Material and Technique:

Since November 1945, all the defective eggs candled out have been opened and their contents thoroughly examined. Altogether 297 such eggs formed the basis on which this study was made.

A record has been kept of the gross appearance and location of all blood and meat spots encountered.

To determine the actual position of the blood spot the yolk with the blood spot on it was carefully separated from the albumen and after being agitated for a few minutes in 0.8% normal saline was immediately fixed in Formol Saline. Dehydration was performed as usual by passing through upgraded alcohols; cedarwood oil was used as/-

as a clearing agent. The yolk was then embedded in toto in paraffin but for histological examination only the portion with the blood spot on it was used to prepare sections.

Similarly the coloured and white meat spots were also fixed and prepared for sectioning. During the earlier part of the investigation the fixatives used were mainly Bouin's and Formol Saline, but later Flemming - without acetic was also employed. Direct study of normal and degenerating yolk immersed in a drop of 2 per cent osmic acid was also made.

Sections were cut 5-6 μ thick and, in general, stained with Delafield's haematoxylin followed by alcoholic Eosin. The following stains were also used: 0.5% Iron-haematoxylin, Thionin blue, Mucicarmine, Mucihaematin, Giemsa's, Mann's and Wiegert's Fibrin stain. Prussian blue and Feulgen's reactions were employed to detect the presence of iron and nuclear chromatin respectively in the white meat spots.

In/-

In order to overcome the existing confusion with regard to the classification of foreign substances found in the hen's egg, all the blood formations (dots, streaks, sheets and clots etc.) which are in close adherence to the vitelline membrane have been included under the term "blood spot". The term meat spot has been reserved for all the discrete solid substances lying either free in the egg albumen or entangled in the chalazae.

Morphology of Blood Spots

The blood spots varied greatly in size and shape. (Plate I figs. 1-5). The smallest of them is no larger than a pin-prick and it is extremely difficult to identify such faults by candling. In some eggs, however, the blood spot is so large as to cover almost the whole yolk (Plate I, fig. 1).

The blood spots are usually in the form of sheets or streaks adherent to the yolk. The sheets may be thin or thick, the latter being darker in colour than the former. Sometimes they are very faint/-

faint, discontinuous streaks (Plate I, fig. 3), but they may also be quite thick and distinct. (Plate I, fig. 2). In some cases the blood spot is in the form of a thick and rounded clot intimately attached to the yolk (Plate I, fig. 4).

Very often it is seen that it is situated on that pole of the yolk which is towards the broad end of the egg. Occasionally associated with such a blood spot can be seen a blood streak which may be either narrow or broad. (Plate I, fig. 5). This streak may or may not end in a large blood clot but it is usually surrounded by the fibres of the chalaza. Consequently the blood streak also reveals in some cases the typical twisted appearance of a chalaza.

Figure I (Plate II) shows the section of a blood spotted yolk. The spot is in the form of a sheet and can be seen lying in between the two membranes covering the yolk. Figure 2 (Plate II) reveals a portion of the same section under higher magnification/-

magnification . Here can be easily seen a mass of blood cells separated from the yolk by means of the vitelline membrane. The outer membrane covering the blood cells is identified as the chalaziferous layer. Most of the blood cells at this stage are apparently quite normal as their nuclei and cytoplasm take up the usual blood stains (Plate II, figs. 3 and 4). A few of them, however, are degenerate and are represented only by their nuclei.

Morphology of Meat Spots

Meat spots also show great variation in size, shape and colour. They may be found in any part of the egg albumen, lying either free or entangled in the fibres of the chalazae (Plate III, figs. 1-3). The smallest of them may be of such an order that it can easily be overlooked by the naked eye in an opened egg, but the largest could readily be distinguished by candling through the shell by even an inexperienced observer. They are usually rounded in shape but may be angular and sometimes elongated. The colour is generally dark red but they may/-

may be red, liver-like or of various shades of brown. Some of them are perfectly white or white with some red or brown specks included.

In figure 1 (Plate IV) a section of a red meat spot is shown. It reveals a central mass of blood cells surrounded by a fibrous layer. Figure 2 (Plate IV) depicts a portion of the same section under higher magnification. Here the granular protein present in between the central mass of blood cells and the outer fibrous layer is seen infiltrating into the mass of blood cells; the blood cells are still normal except that their cytoplasm does not take up any stain. This, however, is not a general rule but depends rather upon the state of degeneration of the blood cells in particular cases. In figure 3 (Plate IV) a section of another red meat spot is shown in which some of the blood cells are quite normal but the cytoplasm of others does not stain.

With the progress of degeneration the meat spots show extensive vacuolization due to the complete/-

complete disintegration of blood cells some of which are, at this stage, represented by their nuclei only (Plate V, fig. 1). These nuclei have a tendency to group together and can be seen in the section of a white meat spot with a red speck in the centre (Plate V, fig. 2). A piece of the white portion of this meat spot is shown under high magnification (Plate V, fig. 3), and reveals it to be made up of coagulated albumen in which are seen masses of degenerating yolk in addition to the blood cells. A little albumen is invariably coagulated outside the fibrous layer surrounding the coloured meat spots and very often normal and degenerating blood cells can be seen in it (Plate IV, fig. 4). Degenerating yolk can at times be identified in the coagulated albumen and even entangled in the constituent fibres of the layer surrounding the coloured meat spot (Plate V, fig. 4).

Generally white meat spots have as their basis such a coagulated mass of albumen but they may show great variation with regard to the amounts of albumen, degenerating blood cells and yolk they contain.

In/-

In a perfect white meat spot, however, there are few, if any, blood cells, and these, if present, are in an extremely degenerate condition. The bulk of a white meat spot consists of coagulated albumen mixed with yolk in various stages of degeneration (Plate VI, fig.1).

It is not uncommon to observe a few cells with extreme vacuolation of the cytoplasm in a white meat spot but it becomes increasingly difficult to identify these in view of the general degeneration in progress. Even broken egg membranes are sometimes included in white meat spots.

Origin of Blood and Meat Spots.

In general, blood spots are structurally composed of a mass of blood cells lying in between the two membranes enveloping the yolk. The inner covering is the vitelline membrane and the outer the chalaziferous layer.

As early as 1898, Mitrophanow also noticed the presence of blood cells in the thickness of the envelope of yolk (enveloppe du jaune) which, according to him, consisted of two layers namely the external or the albuminous layer and the internal or the vitelline/-

vitelline membrane. This albuminous coat of the yolk envelope may be identified as the chalaziferous layer.

It has been shown in this study that the coloured meat spots also are encapsuled by a fibrous layer which stains exactly like the chalaziferous layer. It seems highly probable, therefore, that the chalaziferous layer of the ovum and the fibrous covering of coloured meat spots are similar structures and secreted in the same region of the oviduct.

During the course of this study evidence has been obtained that the chalaziferous layer is secreted by the posterior half of the infundibulum to which the name "chalaziferous region" was given by Richardson (1935). It can, therefore, be inferred that blood spots and coloured meat spots are present before the egg reaches this region of the oviduct, and in consequence the lower portion of it can safely be excluded as the source of blood and coloured meat spots.

This conclusion is further supported by the fact that in most of the double yolk blood spotted eggs the/-

the blood spot is present on one yolk only.

It was not uncommonly held that blood spots are formed as a result of haemorrhage at the time of ovulation following the rupture of a small blood vessel near the stigma. However, the observations on ovulation in timed laparotomized hens made by Nalbandov and Card (1944) have shown that bleeding may occur at this time but it is so rare and insignificant a phenomenon that it cannot be held to be an important agency in producing blood spots in eggs. They further state that the bleeding is intrafollicular and that it may occur several days before ovulation. Though they have never observed active intrafollicular bleeding in laparotomized hens they have noted follicles containing both small and large submembranous haemorrhages in ovaries both excised and in situ. They have also observed the yolks being released with both small and large blood clots adhering to them although there was no active bleeding at the time of ovulation.

Experience derived from the present investigation confirms the findings of these workers as/-

as follicles were encountered containing submembranous haemorrhages (Plate VI, fig. 2). How extensive intra-follicular bleeding can be was shown by an abnormal egg laid by a Brown Leghorn hen of the flock at the Institute of Animal Genetics. In this egg, instead of a normal yolk there was a complete follicle, surrounded by the usual layers of albumen, shell membranes and shell. The follicle appeared to be filled with blood and very little yolk could be detected. (For a detailed description of this egg see Part 4 of this thesis).

As already pointed out by Nalbandov and Card (1944) bleeding may occur anywhere in the follicle. If it is slight the blood remains in between the vitelline membrane and the follicle and thus forms a small blood spot on the former. However, if the bleeding is copious the whole of the vitelline membrane may be smeared with blood and the blood may even accumulate in the pedicel and its funnel-shaped base. This results in the formation of a blood streak with or without a blood clot at its tip, and remains attached to the main blood spot on the vitelline membrane.

Since/-

Since many of the formations of this type are seen on that pole of the yolk which is towards the broad end of the egg (Plate I, fig. 5) and most of the eggs are laid with pointed end first, the hypothesis stated above seems to be quite acceptable. This is further supported by observations on the abnormal egg, referred to above, in which the pedicel contained a blood streak within it.

Frequently during ovulation this blood streak, with or without a clot at its tip, loses its connection with the main blood spot on the vitelline membrane and may become an inclusion in the albumen of the same egg or of subsequent ones.

When such a blood clot reaches the "chalaziferous region" of the oviduct it is surrounded by the fibrous layer secreted there and thus forms a meat spot. If this remains lying in the oviduct then, simultaneously with the degeneration of the blood cells, the adherent albumen is also coagulated on its surface giving it a whitish appearance. In these meat spots, however, reddish specks can still be demonstrated in the centre.

According/-

According to Nalbandov and Card (1944) all meat spots, including white ones, are merely degenerated blood clots resulting from changes in pH of the albumen and that the transformation from red to white meat spot is hastened by high environmental temperatures. It was proved experimentally by using artificial blood clots in albumen and also in buffer solutions of known pH exposed to temperatures of $50^{\circ}F$ and $88^{\circ}F$.

A repetition of their experiments carried out at Edinburgh at a temperature of $103^{\circ}F$ did not change an artificial blood clot into a white meat spot. Blood and meat spots from infertile eggs which had been incubated more than 7 days were still not white. The colour of the meat spots in such eggs changed from red to dark red, brown or even tan but never to white.

Artificial and natural blood clots kept in 0.8 per cent normal saline changed their colour to pinkish white leaving the medium reddish even at ordinary room temperature after a few days. However, on sectioning these and also the artificial and natural blood/-

blood clots and coloured meat spots which had been in the incubator for more than a week the same appearance was not obtained when compared with the section of a white meat spot. In all sections from the former blood cells in various stages of degeneration could always be seen.

A typical white meat spot, as far as the observations go, is formed in the oviduct by the coagulation of the albumen around free yolk left degenerating in the oviduct by the rupture of the membranes surrounding it. Pieces of the broken membranes are sometimes visible as inclusions in white meat spots.

Frequently blood cells and also some other cellular elements can be detected in white meat spots but, because of the extensive degeneration it becomes increasingly difficult to identify them. It is quite possible that these cells may be derived, either from the follicular epithelium or from the developing germ of a yolk which has ruptured in the oviduct.

Lucas/-

Lucas (1946) has described macrophages and fibroblasts in addition to intravascular cell types from smears of blood spots. Though it has not been possible to confirm his findings many of the weird structures figured by him have been noted in sections of white meat spots. It is clear that the problem of white meat spots is one that requires further evidence from an experimental approach towards its solution.

The so-called "bloody white" appears to be formed by the dissolution of a blood clot as frequent observations have shown that the albumen in the proximity of a large blood spot or a red meat spot is blood coloured. Sometimes it is even possible to detect blood cells in such albumen under the low power of a microscope. This was further substantiated by observations of artificial blood clots which dissolve out, sometimes completely, leaving the medium reddish.

It is also possible that the "bloody white" may be produced following haemorrhages from the wall of/-

of the oviduct. Nalbandov and Card (1944), however, could not succeed in producing "bloody white" by injecting blood in the magnum region of the oviduct.

The so-called "cloudy white", according to Nalbandov and Card (1944) results from the bloody white after suitable pH changes, provided the temperature is favourable. It appears more probable, however, that the cloudy white is produced by the disintegration of the chalazæ and the dispersion of the free yolk granules.

Summary/-

Summary and Conclusions.

1. Blood spots in eggs are due to intra-follicular bleeding. If the haemorrhage is only slight it results in the formation of thin sheets and streaks of blood in close adherence to the vitelline membrane. Copious bleeding, on the other hand, leads to the formation of large blood clots which, if separated from the yolk, are included in the egg albumen and form coloured meat spots.

2. White meat spots are formed in the oviduct by the coagulation of albumen around a degenerated coloured meat spot, degenerating yolk, or even broken egg membranes.

3. The so-called "bloody white" appears to be due to the dissolution of a large blood spot or a coloured meat spot.

4. Cloudy white may be formed by the disintegration of the chalazae and the dispersion of degenerating free yolk granules.

Explanation of Plates
Plate I.

Various Types of Blood Spots.

Fig. 1. A very large blood spot covering almost the whole yolk.

Fig. 2. Blood spot consists of distinct sheets and streaks adherent to the yolk. The blood streak towards the left (broad end of the egg) is continued into the albumen and ends in a large dark red blood clot.

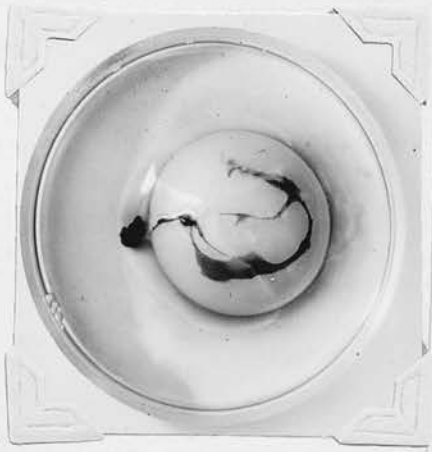
Fig. 3. Blood spot consists of faint and discontinuous streaks on the yolk.

Fig. 4. Blood spot consists of a dark red thick and rounded blood clot intimately attached to the yolk. Two small blood dots can also be seen near it.

Fig. 5. The blood sheet is situated on that pole of the yolk which is towards the broad end of the egg. It is continued into the albumen in the form of a thick and dark red blood streak and ends into a large blood clot of the same colour. The blood streak presents a typical twisted chalaza-like appearance due to the chalazal fibres surrounding it.

Plate I

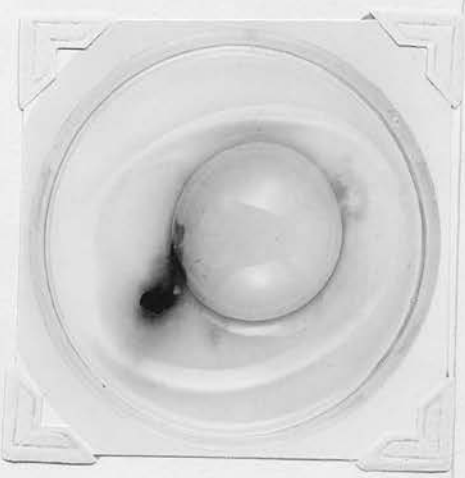
1



2

3

4



5

Plate II.

S E C T I O N S.

Unless otherwise mentioned, all sections have been stained with Delafield's haematoxylin and eosin.

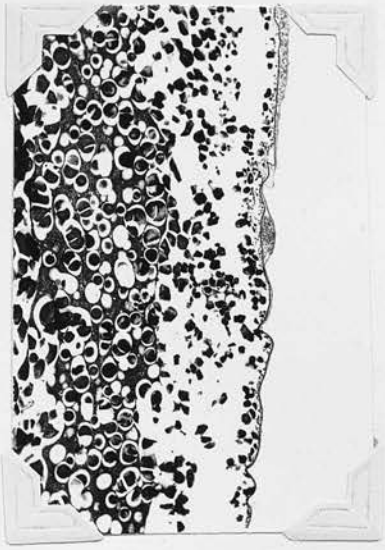
Fig. 1. Section of a blood spotted yolk. X 40.

Fig. 2. A portion of the same section. X 350.

Fig. 3. Section of another blood spotted yolk. X 350.

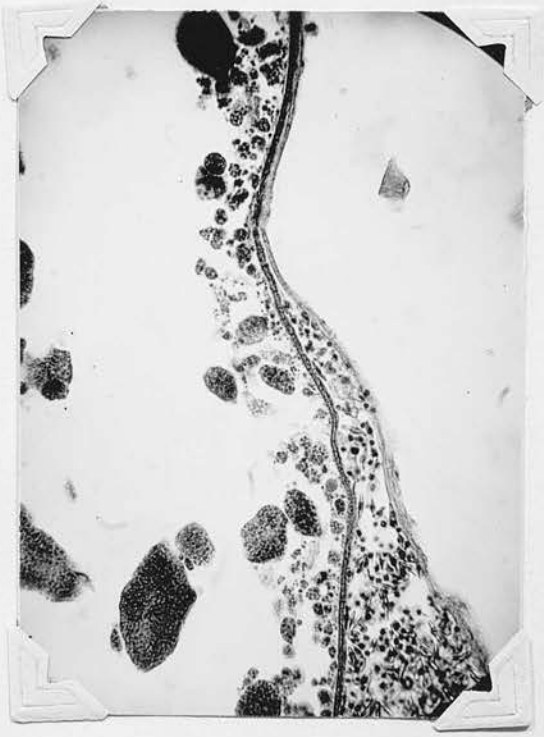
Fig. 4. Section of a blood spotted yolk stained with Thionin. Here the chalaziferous layer stains purplish pink while the vitelline membrane is pale blue. X 700.

Plate II.



1

2



3

4

Plate III.

Various types of meat spots.

Fig. 1. A red meat spot entangled in the free end of the chalaza.

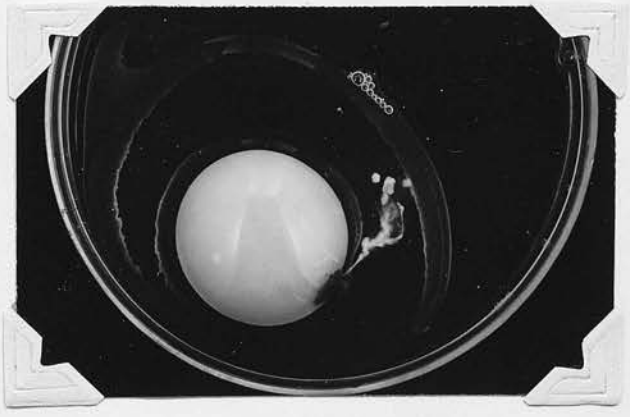
Fig. 2. Meat spots of various colours in the albumen and two small blood streaks on the yolk.

Fig. 3. White meat spots in the albumen and a blood sheet on the yolk.

Plate III

1

2



3

Plate IV.

S E C T I O N S.

Unless otherwise mentioned, all sections have been stained with Delafield's haematoxylin and eosin.

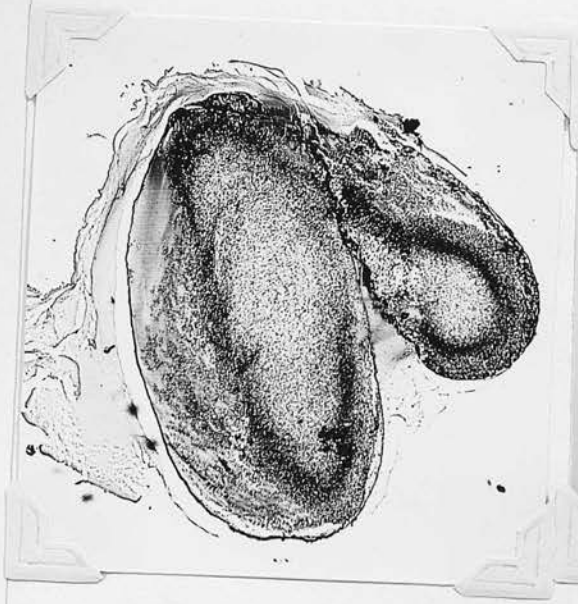
Fig. 1. Section of a red meat spot stained with Delafield's haematoxylin and muci-carmine. X 40.

Fig. 2. A portion of the same section. X 350.

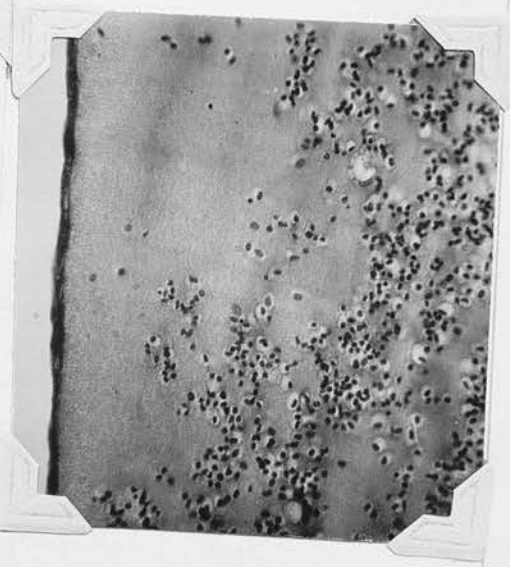
Fig. 3. Section of another red meat spot. Some of the blood cells are quite normal. X 350.

Fig. 4. Section of a red meat spot where the blood cells can even be seen outside the fibrous layer embedded in the coagulated albumen. X 350.

Plate IV

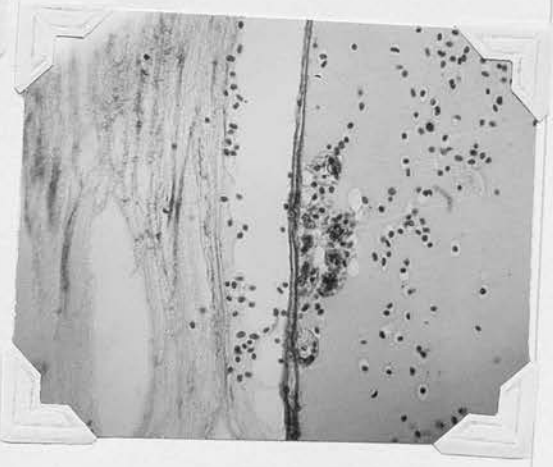


1



2

3



4

Plate V.

S E C T I O N S.

Unless otherwise mentioned, all sections have been stained with Delafield's haematoxylin and eosin.

Fig. 1. Section of a dark red meat spot showing extensive vacuolization due to the disintegration of blood cells. 0.5% Iron haematoxylin and eosin. X 45.

Fig. 2. Section of a white meat spot with a red speck in the centre. X 40.

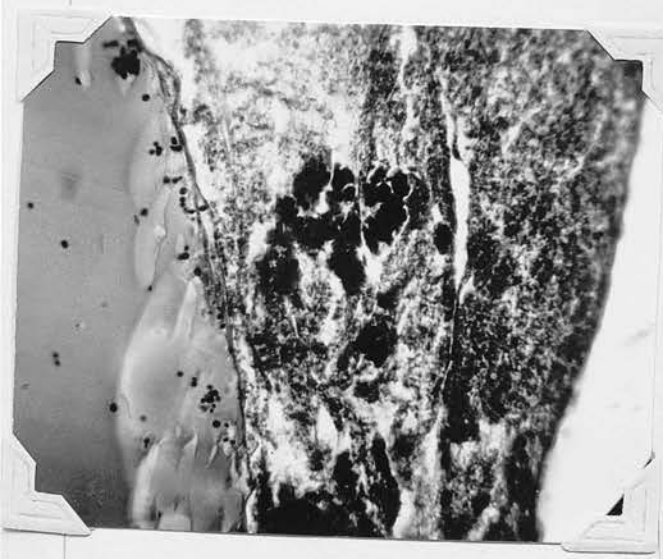
Fig. 3. A portion of the same section. X 350.

Fig. 4. Section of a tan-coloured meat spot from an infertile egg which had been incubated for nearly two weeks. X 300.

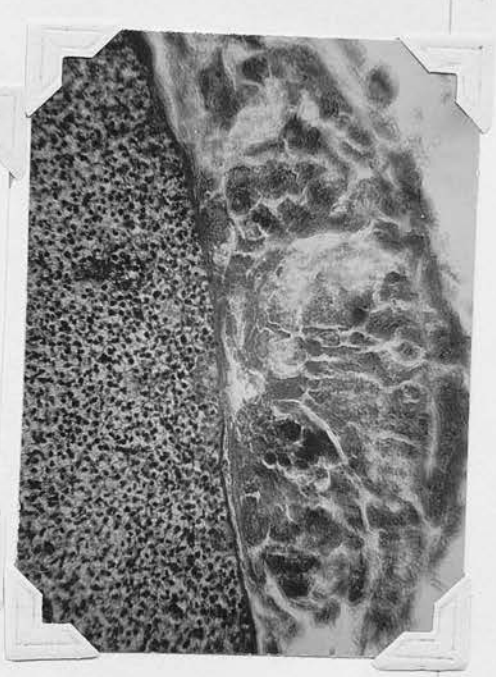
Plate V

1

2



3



4

Plate VI.

Fig. 1. Section of a white meat spot. X 20.

Fig. 2. Excised ovary showing submembranous haemorrhages in the follicles.

Plate VI

1

2

Part II.

Section B. Structure and Formation of Miniature Eggs of the Domestic Fowl.

INTRODUCTION:

In a comprehensive paper Pearl and Curtis (1916) discussed various aspects of the miniature or "dwarf" egg. The shape of such eggs was classified as either prolate-spheroidal or cylindrical, the latter being quite rare. An examination of the contents revealed that 35.04% of such eggs were yolkless, 55.11% contained free yolk and that only 9.85% had the yolk enclosed in a membrane.

Crew (1930), in a short note to the "Feathered World", confirmed in general these conclusions.

Asmundson (1931) has stated that dwarf eggs are produced as a result of the ovulation of yolks which subsequently escape into the body cavity of the bird intact or following rupture of the vitelline membrane.

Because of the suggested existence of yolk-less miniature eggs special attention was given in the/-

the present investigation to the nature of the stimulus leading to the secretion of albumen in such eggs.

Material and Methods

All miniature eggs laid by the Institute flock during the period January 1946 to the end of February 1947 were weighed and, in addition, an index of shape determined by dividing maximum length by maximum breadth. They were then opened and the contents carefully scrutinised. The non-albumenoid part of the egg content was agitated in 0.8% normal saline and then prepared after fixation in Bouin's fluid, Formol saline or Flemming - without acetic, for histological examination. Sections 5-6μ thick were stained with Delafield's haematoxylin followed by alcoholic eosin. Thionin blue and 0.5% Iron-haematoxylin were also employed on occasions.

For the study of yolk granules fresh material was immersed in a drop of normal saline to which 2% osmic acid was added.

Results/-

Results and Discussion

A. The Analysis of Shape.

In figures 1 and 2 (Plate I) five miniature eggs are shown with a normal egg for comparison. The larger egg in figure 2 is definitely cylindrical in shape and the others would be described as prolate-spheroidal under Pearl and Curtis' classification since none of them differs markedly from the typical egg shape. Measurements of the ratio, length/breadth, for 99 miniature eggs gave values varying from 1.11 to 1.60, a range quite usual in the ordinary production records of the flock. Their distribution in regard to shape, however, differed somewhat from that for normal eggs as is demonstrated in Table 11 where their frequency in six classes is compared with the distribution of percentages of a random sample of 1083 eggs from the 1946 flock.

Table 11/-

Table 11.

Distribution of Miniature and Normal Eggs in Regard to Shape.

Length/breadth ratio.	1.00-1.19	1.20-1.29	1.30-1.39	1.40-1.49	1.50-1.59	1.60 - 169
Miniature Eggs Number	21	54	20	2	1	1
Normal Eggs Percentage	0.46	32.87	52.72	12.10	1.57	0.28

Relatively more miniatures fall into the first two classes and they are obviously rounder on the average suggesting that there may be some relationship between shape, as measured by this index, and the size of the egg. This, at first glance, appears to be confirmed by the comparison of egg weight and mean index in Table 12 but it is clear that the range of indices within the various weight classes is sufficiently wide to offset the validity of such a conclusion.

Table 12

Weight and Index (Length/Breadth) Range of Miniatures.

Class	Population.	Mean Weight	Mean Index	Range
50-59 gm.	1	53 gm.	1.36	
40-49 gm.	9	44 gm.	1.28	1.33-1.22
30-39 gm.	21	35 gm.	1.29	1.37-1.19
20-29 gm.	19	24 gm.	1.25	1.43-1.14
10-19 gm.	39	14 gm.	1.24	1.60-1.11
0-9 gm.	10	6 gm.	1.22	1.32-1.13

B./-

B. Contents of Miniature Eggs.

With regard to the non-albumenoid contents of miniature eggs most previous workers have classified them as (1) yolkless, (2) with free yolk not enclosed in a membrane, or (3) with a typical yolk much reduced in size. Out of a total of 100* eggs used in this study, 85 were found to contain yolk in one form or another; and of these 12 had spherical yolks with the usual membranes intact. The weight and index of these eggs is given in Table 13.

Table 13/-

* One of the miniature eggs examined could not be included in Tables 11 and 12 because no shell had been secreted when the egg was laid.

Table 13.

Yolked Miniatures.

Weight in gm.	49.1	46.2	43.4	38.1	38.0	36.4	35.5	28.3	28.2	25.7	23.6	11.7
Index(L/B)	1.33	1.28	1.24	1.26	1.30	1.26	1.31	1.23	1.26	1.43	1.21	1.24

Seven more had normal yolks but in addition they revealed some free yolk dispersed in the albumen. The presence of this free yolk in the albumen can be explained as follows:- Either it came from the yolk left degenerating in the oviduct by the rupture of the membranes of a previous egg or the membranes of the same egg ruptured sometime during its passage through the oviduct and later on closed leaving some free yolk in the albumen.

Eighteen eggs contained some yolk covered by broken membranes (Plate II, fig. 1); in one of these the inclusion was quite small and it appeared to be a fragment of free yolk but on microscopical examination broken vitelline membrane could be seen surrounding it. In addition to yolk in broken membranes seven eggs showed a few fragments of free yolk in the albumen (Plate II, fig. 2) while in eleven a further dispersal of tiny pieces of free yolk through the thick albumen had taken place as illustrated in figures 3 and 4 (Plate II).

In 27 eggs the whole of the thick albumen appeared/-

appeared yellowish due to the distribution of free yolk within it. The albumen seemed to function as a containing envelope for when it was punctured pale yellow, or yellowish white, thin albumen began to flow out carrying with it yellow and whitish masses. The latter, in most cases, appeared to consist of remnants of broken membranes while the colour of the albumen was undoubtedly due to the presence of yolk granules in it.

Of the three eggs in which the nucleus was formed by free yolk only two contained a single piece of yolk material. In one of these the piece of yolk was so small that it could be easily missed. In the other it was quite large and the albumen of the egg was also coagulated around it. The third egg seemed to be a double egg. It weighed only 3.66 grammes and, so far as the shape was concerned it was the miniature replica of a normal egg. In the centre it contained an oval white opaque substance surrounded by a solid layer of thick albumen. The outer layer of thin albumen was normal and so were the shell membranes and the/-

the shell. The central inclusion had on its surface a number of tiny pieces of free yolk. When it was punctured a whitish cord formed by a collection of mucin fibres, in which yolk granules were also entangled, was revealed. This cord was also surrounded by albumen. The external envelope of the central opaque substance seemed to be a thickened shell membrane.

In Plate III are shown the sections of a number of yolks surrounded by broken membranes. When a portion of figure 1 (Plate III) is seen under higher magnification (Plate IV, fig. 1) it is noticed that the yolk is surrounded by two membranes. The outer one is the chalaziferous layer and the inner, which is thrown into folds, is the vitelline membrane. It appears, therefore, that the vitelline membrane in this case ruptured before the normal yolk reached the "chalaziferous region" of the oviduct. The folds in the vitelline membrane could be produced as a result of the flowing out of the greater part of the yolk and later on the chalaziferous layer was secreted round the folded vitelline membrane covering the remains of the yolk.

In/-

In figure 2 (Plate IV) which represents a portion of figure 4 (Plate III) under higher magnification, only the folded vitelline membrane is seen covering the degenerating yolk. There is no definite chalaziferous layer but a few mucin fibres can be seen running into the folds of the vitelline membrane. It is quite possible that the rupture in this case may have taken place after the secretion of the chalaziferous layer had started and so the mucin fibres of the chalaziferous layer also are drawn into the folds of the vitelline membrane.

The albumen of an egg is very often coagulated on such a mass of yolk covered by broken vitelline membrane giving it a whitish appearance. The thickness of such a layer of albumen varies in different eggs as will be clear from figures 2, 3 and 4 (Plate III).

In the same way the albumen is coagulated on pieces of yolk set free in the oviduct by the rupture of the membranes covering it. One such piece is shown in figure 3 (Plate IV) and a portion of it under higher magnification is shown in figure 4 (Plate IV). Here

one finds no membranes separating the yolk from the coagulated egg albumen. Likewise the membranes also from which most of the yolk has already been set free are sometimes covered by the coagulated albumen (Plate V, fig. 1). Two portions of the same section under higher magnification are shown in figures 2 and 3 (Plate V). In figure 3 (Plate V) the yolk is seen degenerating inside the membranes while in figure 2 (Plate V) the degenerating yolk is embedded in the albumen coagulated on the mass of broken membranes. Such masses of free degenerating yolk in the coagulated egg albumen lead to the formation of white meat spots, very often met with in miniature eggs.

Out of 15 eggs which did not show any apparent yolk, 13 contained some sort of central mass around which the albumen was secreted. In most cases this consisted of tiny white meat spots.

In 6 eggs, in addition to the white meat spots, the central mass revealed white convoluted cords which could be easily mistaken for chalazae (Plate VI, fig. 1). These cords showed certain thickenings at intervals and on histological examination were found to contain

degenerating/-

degenerating yolk mixed with coagulated albumen. The cords appeared to be made up of mucin fibres.

Five of these eggs contained a whitish central mass. This consisted of coagulated albumen and clusters of mucin fibres. Tiny white meat spots were invariably entangled in these masses.

In one egg the nucleus was formed by a cream coloured mass situated in a whitish area of the thick albumen (Plate VI, fig. 2). On histological examination this cream coloured mass revealed yolk granules entangled in the mucin fibres of the coagulated albumen.

The contents of another egg are shown in fig. 1 (Plate VII). Here, in addition to the white meat spots, a piece of membrane (possibly vitelline membrane) was also included in the albumen. In figure 2 (Plate VII) is shown a tiny white meat spot from the same egg under low magnification. A few of the yolk granules forming this white meat spot are seen under higher magnification in figure 3 (Plate VII). Such pieces of degenerating yolk are present in most white meat spots. Even the albumen of the two eggs which

did/-

did not show any central mass revealed such degenerating yolk granules. Sometime one can see these degenerating yolk granules entangled in the mucin fibres of the chalazae even in a normal egg (Plate VII, fig. 4).

C. The Nature of the Albumen in Miniature Eggs.

As has already been pointed out by Pearl and Curtis (1916) miniature eggs differ with regard to the density of the albumen. In most of them all the layers of albumen (inner thin, thick and outer thin) could be easily made out as in a normal egg. In some miniature eggs, however, the inner and the outer thin albumen were absent, the central mass being surrounded by a layer of thick albumen only. In such cases the albumen was very much condensed and appeared like that of a normal egg when it is in the magnum region of the oviduct.

In some miniature eggs where the central mass is not a normal yolk, the albumen immediately surrounding the central mass appears whitish. Similarly the albumen surrounding the free pieces of yolk or sometimes even the while meat spots appears whitish/-

whitish. This whitish area in some cases appeared to be merely coagulated albumen but in others it revealed tiny free yolk granules dispersed through it.

D. Factors responsible for the Production of Miniature Eggs:

Pearl, Surface and Curtis (1911) have stated that the factors involved in the production of miniature eggs are:

1. "The bird must be in an active laying condition; the more pronounced the degree of physiological activity of the oviduct the more likely are these eggs to be produced".

2. "There must be some foreign body, however minute, to serve as the stimulus which shall start the albumen glands secreting. This foreign body may be either a minute piece of hardened albumen, a bit of coagulated blood, a small piece of yolk which has escaped from a ruptured yolk, etc.".

3. "It seems likely, though this is a point not yet definitely settled, that ovulation (i.e. the separation of a yolk from the ovary) must precede the secretion of albumen around the foreign body to form one/-

one of these eggs".

Pearl and Curtis (1916) later confirmed these conclusions. They autopsied five of the eleven birds which produced few or no normal eggs after the miniature egg or eggs and found that -

1. "Each bird was a normal high laying individual which became unable to produce normal eggs on account of a pathological condition* of the oviduct".
2. "In every case the part of the duct affected was the posterior end of the funnel or the anterior end of the albumen secreting region or both".
3. "The disturbance in each case was of a nature to constrict or prevent the normal expansion of the lumen of the duct."
4. "In no case was the passage completely closed".
5. "In each case there was convincing evidence that the ovary was in a normal reproductive cycle at the time the dwarf egg was produced".

It/-

* Four of the five cases had tumorous growths on the walls of the duct. In two cases these involved a large part of the funnel and albumen secreting region. In the other two the affected tissue was confined to a narrow band in the lower funnel or albumen secreting region. In the fifth case there were two constrictions of the duct in the anterior end of the albumen secreting region. The constrictions were separated by one centimeter of duct with a normal diameter. The tissue in these constrictions did not appear pathological.

this particular region, in which event the chalaziferous layer would be secreted round the folded vitelline membrane covering the remains of yolk, or the "chalaziferous region" is defective and no chalaziferous layer is secreted. Consequently the delicate vitelline membrane which by itself is unable to bear the pressure of the contracting muscles of the oviduct would rupture. That this is actually so has already been shown by the autopsy records of Pearl and Curtis (1916). It will not be out of place to mention here the work of Burmester and Card (1939). They removed 2-7 cm. long sections from the "chalaziferous region" of the oviduct and reported that such resection was conducive to the formation of dwarf or yolkless eggs. It can, therefore, be safely concluded that, due to some abnormal condition of the "chalaziferous region" of the oviduct either no chalaziferous layer is secreted resulting in the rupture of the vitelline membrane under the too great pressure of the contracting muscles of the oviduct or the lumen of the oviduct is/-

is so reduced that it cannot allow a normal yolk to pass through it. In all such cases either no eggs are produced at all or only miniature eggs which can pass through the abnormal "chalaziferous region" are laid. However, the bird still remains in the active laying condition and ova are shed into the body cavity. This fact can be confirmed by the post mortem examination reports of a number of birds from the present flock. For example, hen No. L1178 was hatched on 27th March 1939 and laid her first egg on the 1st October, 1939 when she was 188 days old. She produced 4 miniature eggs in 1943, 13 in 1944, 4 in 1945 and 3 in 1946. She died on 9th June 1946 and the examination post mortem revealed the presence of numerous small egg membranes scattered throughout the abdominal cavity, an indication that the bird must have ovulated frequently. This has also been noted by Pearl and Curtis (1916).

Discussing the nature of the stimulus which leads to the secretion of the various egg envelopes in the oviduct Pearl and Curtis (1916) showed that 64.96 per cent of all the miniature eggs contained yolk/-

yolk in some form or the other. The presence of yolk fragments in such miniature eggs, according to them, may be due to the following causes:
"1. A yolk may have been broken during its passage into the duct and only a part of it may have entered the duct. 2. A part of a yolk ovulated into the body cavity and broken either before or after ovulation may have been picked up by the funnel. 3. A normal yolk may have entered the duct and being unable to pass the pathological portion may have been broken and a part of it extruded into the body cavity. The remaining portion may have passed the obstruction, becoming the effective stimulus for the formation of egg envelopes".

Pearl and Curtis (1916) could not ascertain the effective stimulus in those miniature eggs which, according to them, do not contain any yolk. They, however, noticed some firmer material in the centre of the thick albumen of such eggs. As this firmer material in most cases resembled the fibres of a normal chalaza they suggested that "in some or all of these/-

these cases a normal yolk has entered the duct, stimulated the upper duct to secret chalazae and some albumen, passed as far as the obstruction and then been extruded, leaving behind sufficient chalazae and albumen to furnish the mechanical stimulus necessary for the completion of the egg".

It is true that the central mass in most of the so-called yolkless miniature eggs appears to be like the chalazae but in reality it is not so as it has been shown by various workers that the chalazae do not appear in an egg till it is in the uterus (see also Part 3 of this thesis). This central mass consists of a collection of mucin fibres and very often tiny white meat spots can also be seen entangled in them. These white meat spots have been shown to consist of coagulated albumen mixed with degenerating yolk (see also section A of this part of the thesis). Even in those miniature eggs where no central mass could be seen it has been possible to demonstrate tiny granules freely floating in the albumen. These granules take up a brownish to black tinge when studied under a drop of 2% osmic acid and are obviously/-

obviously yolk. Such granules can also be seen when the remains of yolk from the broken membranes of a miniature egg are studied. In view of these observations it is now suggested that yolk is the only effective stimulus which leads to the secretion of the various egg envelopes in the oviduct. To provide this stimulus the bird must be in the active laying condition and it should be ovulating as has already been pointed out by Pearl, Surface and Curtis (1911), Pearl and Curtis (1916), Crew (1930) and Asmundson (1931).

Summary/-

Summary and Conclusions:

Miniature eggs are either prolate spheroidal or cylindrical in shape, the former being more common. Only four eggs out of a hundred could be placed in the latter group.

Yolk has been shown to be present in the contents of every miniature egg examined and consequently it is considered to be the only effective stimulus which leads to the secretion of various egg envelopes.

The miniature eggs are produced only when the bird is in active laying condition and is actually ovulating.

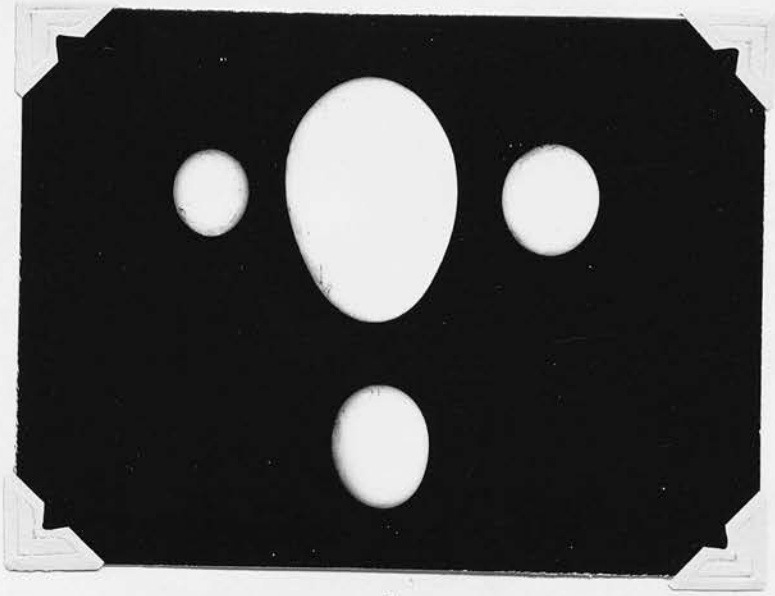
Explanation of Plates

Plate I.

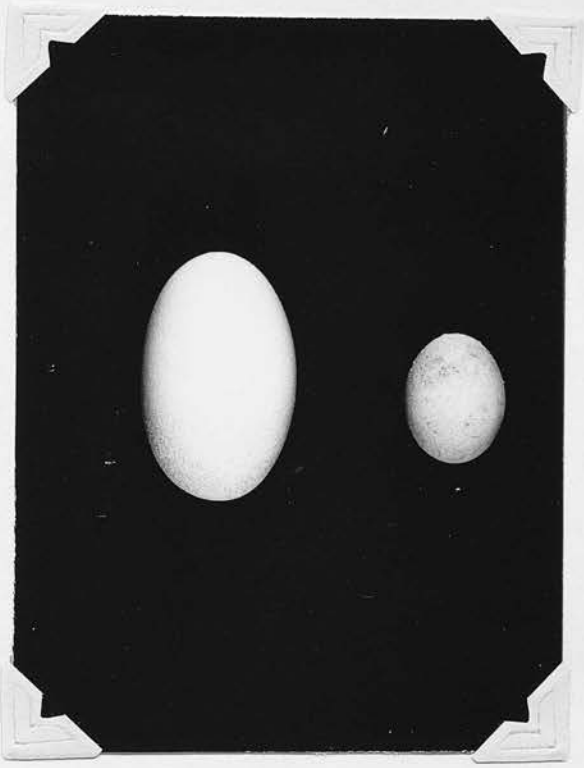
Fig. 1. Three prolate-spheroidal miniature eggs with a normal egg for comparison. X $\frac{1}{2}$.

Fig. 2. Two miniature eggs. One on the left is definitely cylindrical. X 4/5.

Plate I



1



2

Plate II.

Contents of miniature eggs.

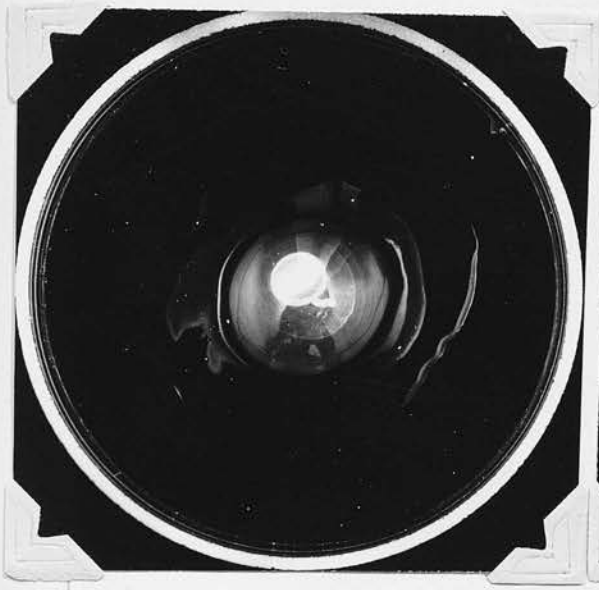
Fig. 1. In the centre is the yolk covered by broken membranes. The dense albumen immediately surrounding it is coagulated to form a transparent capsule. A white meat spot can also be seen outside the capsule.

Fig. 2. In addition to the yolk in broken membranes a few fragments of free yolk are also seen in the albumen.

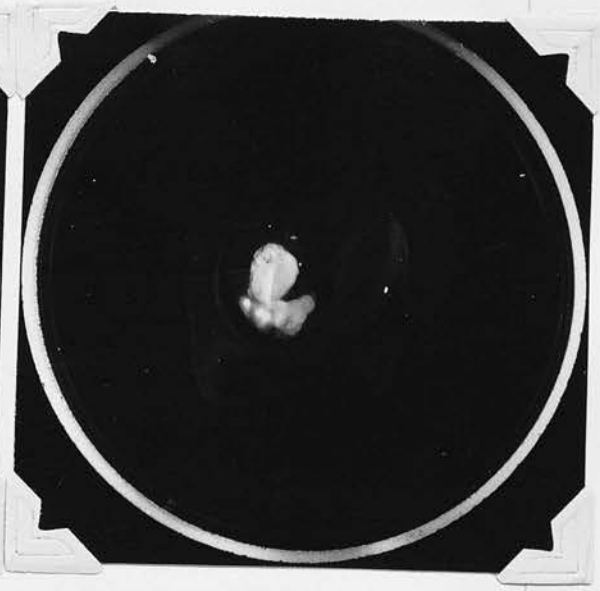
Fig. 3. Broken membranes covering the remains of yolk can be clearly seen in this egg. Pieces of free yolk are seen dispersed through the thick albumen.

Fig. 4. Yolk in broken membranes is represented in this egg by a horse-shoe shaped structure in the centre and a yellowish white piece towards the periphery. The chalaza-like convoluted cord is formed by a collection of mucin fibres. The tiny pieces of free yolk can be seen dispersed through the thick albumen.

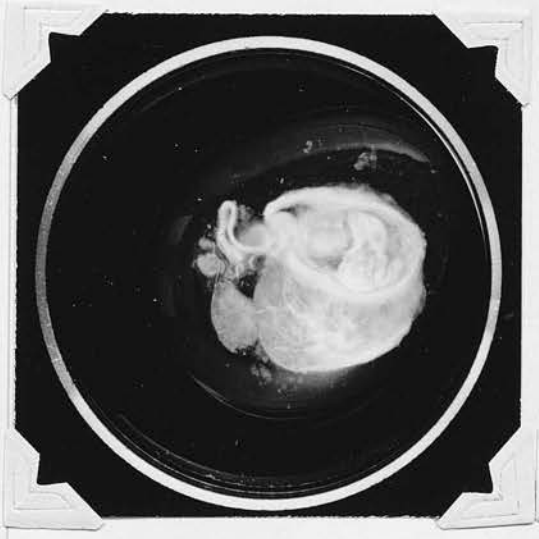
Plate II



1



2



3

4

Plate III.

S E C T I O N S.

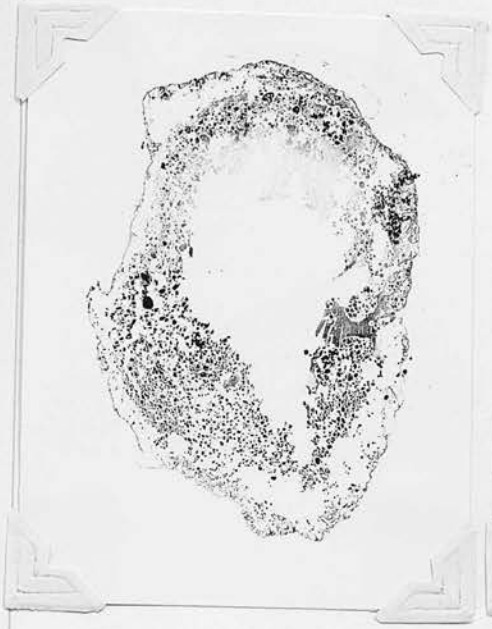
Fig. 1. Section of a yolk covered with broken membranes. X 10.

Fig. 2. Section of a yolk covered with broken membranes. A thin layer of coagulated albumen can be seen at the periphery. X 10.

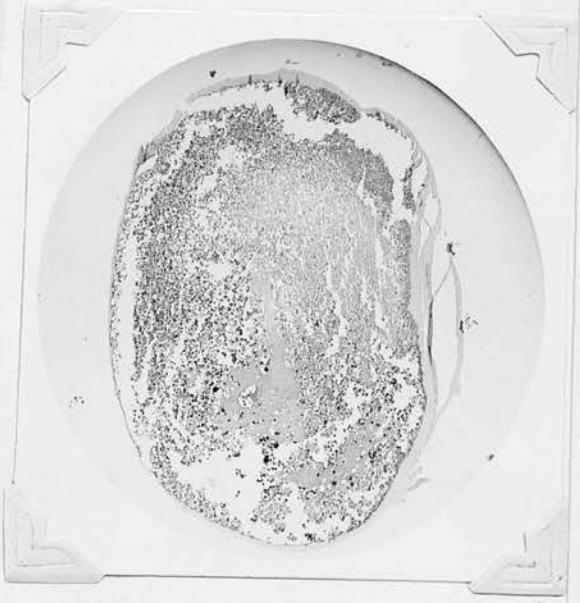
Fig. 3. Section of a yolk covered with broken membranes. The layer of coagulated albumen is quite distinct. X 25.

Fig. 4. Section of a yolk covered with broken membranes. Free degenerating yolk can also be seen outside the membranes embedded in the coagulated albumen. X 15.

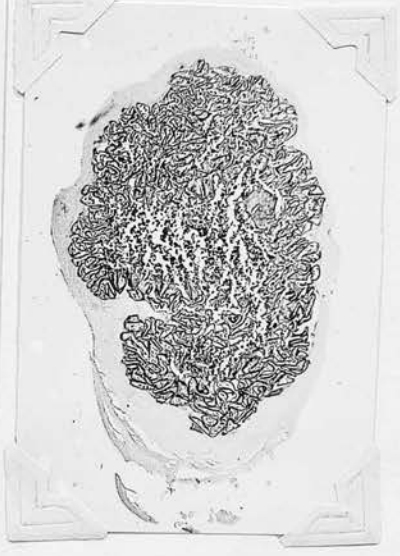
Plate III



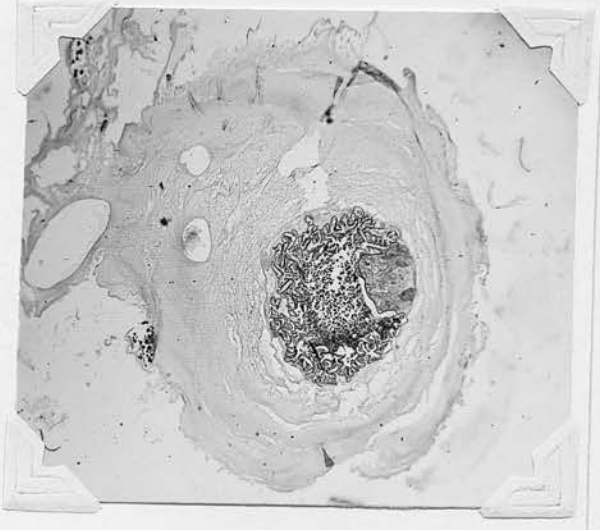
1



2



3



4

Plate IV.

S E C T I O N S.

Fig. 1. A portion of fig. 1 (Plate III). The outer of the two membranes covering the yolk is the chalaziferous layer and the inner, which is thrown into folds, is the vitelline membrane. X 300.

Fig. 2. A portion of fig. 4 (Plate III). The convoluted membrane covering the yolk is the vitelline membrane. There is no definite chalaziferous layer but the mucin fibres can be seen running into the folds. X 300.

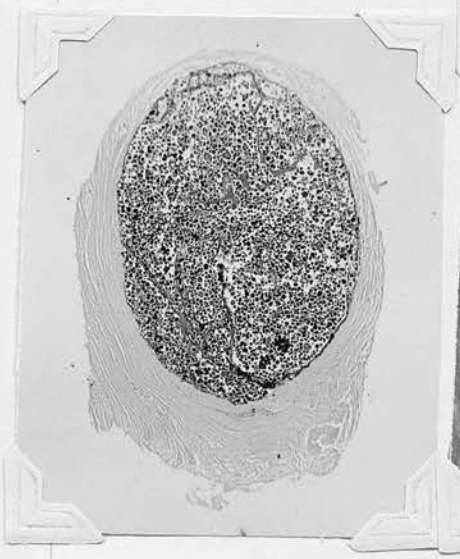
Fig. 3. Section of a free piece of yolk with albumen coagulated on its surface. X 15.

Fig. 4. A portion of the same section. No membranes are seen separating the yolk from the albumen coagulated on its surface. X 350.

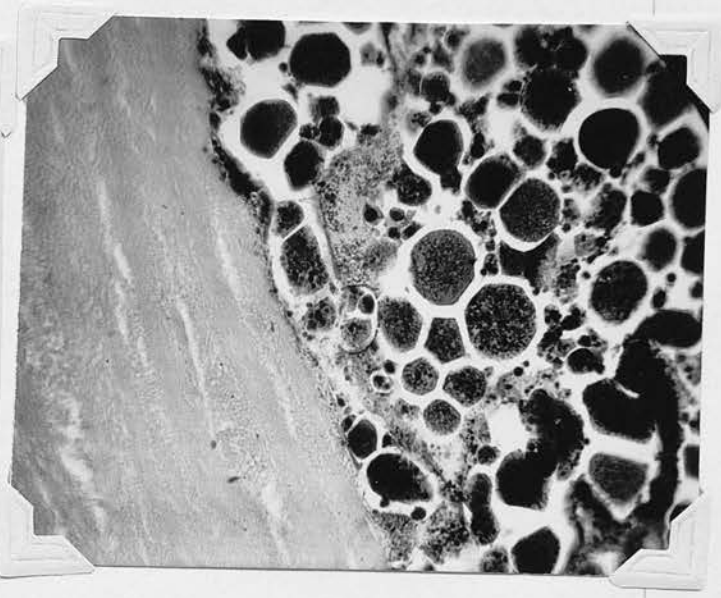
Plate IV

1

2



3



4

Plate V.

S E C T I O N S.

Fig. 1. Broken membranes covered by coagulated albumen. X 15.

Fig. 2. A portion of the same section showing the free degenerating yolk embedded in the albumen coagulated outside the membranes. X 100.

Fig. 3. Another portion of the same section showing the degenerating yolk inside the membranes. X 350.

Plate V

1

2

3

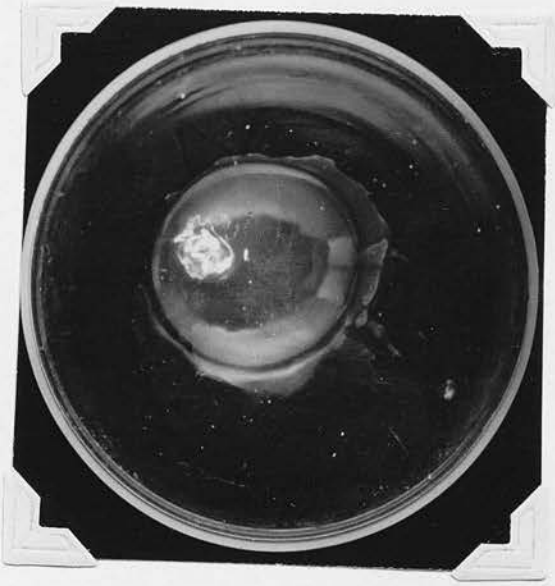
Plate VI.

Contents of miniature eggs.

Fig. 1. The nucleus in this egg is formed by a white chalaza-like convoluted cord showing thickenings at intervals.

Fig. 2. A cream-coloured mass situated in the whitish central area of the albumen forms the nucleus of this egg.
A tiny white meat spot can also be seen in the albumen outside the whitish central area.

Plate VI



1

2

Plate VII.

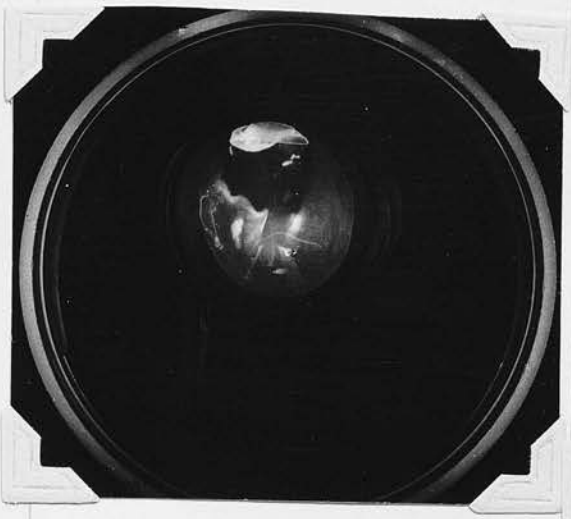
Fig. 1. Contents of a miniature egg showing a piece of membrane (Vitelline membrane?) in addition to a number of white meat spots.

Fig. 2. A tiny white meat spot from the same egg showing the dispersion of degenerating yolk granules through the albumen.

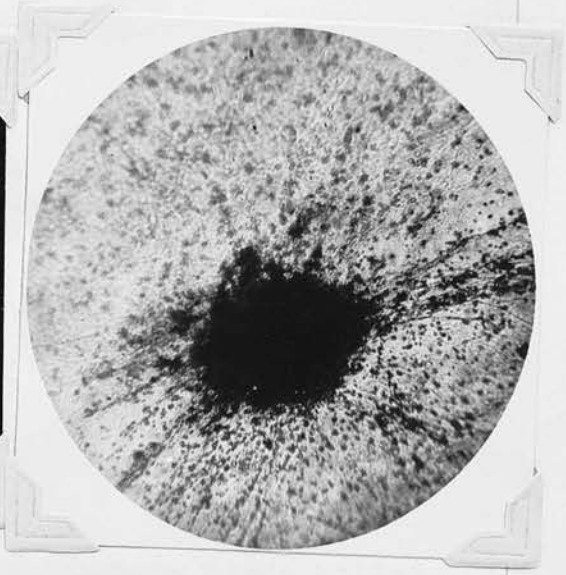
Fig. 3. A few degenerating yolk granules from the same egg (Unstained). X 250.

Fig. 4. A few degenerating yolk granules entangled in the mucin fibres of a normal chalaza (Unstained). X 350.

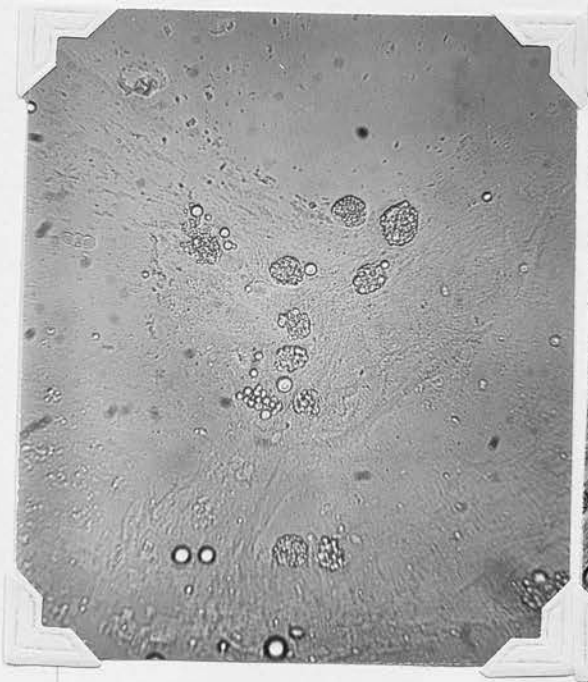
Plate VII



1



2



3

4

Part III.

Some Observations on the Formation of the Hen's Egg with Special Reference to the Chalaziferous Layer and the Chalazae.

Introduction:

In connection with investigations on the origin of blood and meat spots in the hen's egg opportunities arose to examine the ovaries and the oviducts of a number of Brown Leghorn birds killed from time to time at the Institute of Animal Genetics. Occasionally during such examinations immature eggs in various stages of formation from different levels of the oviduct were obtained. On opening some such eggs obtained from the uterus no distinct structure resembling the normal twisted chalazae, so conspicuous in a laid egg, could be found. These observations, in view of the existing confusion in the literature with regard to the formation of the chalaziferous layer and the chalazae led to a more extended and detailed study.

Previous/-

Previous Work.

Pearl and Curtis (1912) stated that the yolk during its passage through the magnum region of the oviduct acquired its chalazae, chalaziferous layer, the dense albumen and also the inner layer of thin albumen. They were, however, doubtful about the existence of the last named layer. The outer layer of thin albumen, according to them, was added in the isthmus and the uterus by osmosis through the shell membranes already formed.

Surface (1912), in his description of the infundibulum has described two kinds of epithelial cells lining the folds of the mucous membrane, namely ciliated and non-ciliated, the former being confined to the more superficial parts of the folds. The non-ciliated cells, according to him, line the deeper parts of the grooves between the folds and he regards these as glandular. Consequently the grooves they line are named by Surface as "glandular grooves" or "gland pouches". He has also distinguished certain other/-

other non-ciliated cells in the region of mergence of the infundibulum into the magnum and has named these as "Unicellular glands".

The presence of glands in the infundibulum led Surface to suggest that the chalazae and the chalaziferous layer are secreted in this part of the oviduct.

Bradley (1928), while confirming Surface's statements, has added that Surface's "unicellular glands" (his goblet cells) produce a secretion which, "from its staining properties, must be looked upon as either mucin or something akin thereto".

Richardson (1935) showed that the chalazae and the chalaziferous layer were secreted in the caudal half of the infundibulum and he called this the "chalaziferous region" of the oviduct.

Hansen (1933) found that in the magnum region of the oviduct only the very viscid layer of albumen was secreted and as he could not see any chalazae till the egg had been in the uterus for some time he concluded that they were formed in the uterus. He also doubted/-

doubted the observation made by Pearl and Curtis (1912) with regard to the taking in of the outer layer of thin albumen as such through the shell membranes. According to him, this layer appears as the result of dilution of the dense albumen with the watery solution of salts diffusing through the shell membranes.

Conrad and Phillips (1938) confirmed Hansen's observations. According to them the layer of dense albumen next to yolk becomes more like a fluid before the egg reaches the uterus. There, due to the rotation of the albumen around the yolk, the mucin of this fluid-like gel is segregated to form the chalazae and the chalaziferous layer, leaving a much more fluid inner thin albumen. They even produced this change experimentally.

Scott and Huang (1941), on the other hand, confirmed Richardson's observation on the formation of the chalaziferous layer. The chalazal formation was first noted by them in the small end of eggs removed from the posterior magnum.

Material/-

Material and Methods

All eggs removed from the various levels of the oviduct were carefully examined for the nature of the albumen and also for the presence or absence of the chalazae. The yolks were then carefully separated from the albumen and after being agitated for a few minutes in 0.8% normal saline, were immediately fixed in Formol Saline. The dehydration was done as usual by passing the specimens through up-graded alcohols and cedarwood oil was used as a clearing agent. The yolks were embedded as such but for sectioning small pieces were cut from the two poles and also from the sides.

Sections were cut 5-6 μ thick and stained with Delafield's haematoxylin and Eosine technique. Thionin blue was also used to differentiate the chalaziferous layer from the vitelline membrane.

Observations:

A casual observation of all the eggs removed from the anterior and mid-magnum region of the oviduct shows the yolk to be surrounded by a very dense/-

dense envelope of albumen only. No definite chalazae can be made out at this stage. Figures 1 and 2 are the photomicrographs of sections of such yolks from the anterior and the mid-magnum region respectively. These clearly reveal two membranes surrounding the yolk. The outer of these is the chalaziferous layer and the inner the vitelline membrane. Both the layers can be easily seen even in preparations stained with Delafield's haematoxylin followed by Eosine. Thionin blue, however, gives the best differential results, staining the chalaziferous layer a purplish pink and the vitelline membrane a pale blue. It is very difficult, however, to preserve this stain during dehydration.

Apparently the eggs from the posterior magnum region of the oviduct are also similar to those described above. However, on closer observation a faint whitish cloud can very often be observed near that pole of the yolk which is towards the isthmus. Figure 3 represents the section of such a yolk from the polar region. Here, in addition to the two membranes covering the yolk one can also see a cluster/-

cluster of fibres outside the chalaziferous layer. These fibres, which constitute the whitish clouds referred to above, stain with all the mucin stains exactly like those of the chalaziferous layer. These are, therefore, nothing but mucin fibres and are the precursors of the chalazae. Simultaneously with the appearance of the mucin fibres the inner layer of thin albumen also becomes visible. It can be made to flow out by puncturing the envelope of thick albumen. Very often the mucin fibres forming the faint whitish clouds also flow out with it.

Figure 4 is a section of that pole of a yolk which was towards the isthmus. When this egg was removed from the oviduct only approximately one half of it was covered by a thin shell membrane while the other half, not yet within the isthmus, was without it. This section has been particularly selected to show the mucin fibres joining the chalaziferous layer at two points. These represent the bases of the chalazal core. Even in laid eggs the bases of the chalazae can very often be seen to form cap-like structures at the/-

the two poles of the yolk.

In the isthmian eggs faint whitish clouds can be made out at both poles of the yolk. The inner layer of thin albumen is also present in these eggs. The thick albumen is still quite dense and no definite outer layer of thin albumen can be seen.

Figure 5 represents the contents of a shelled egg from the uterus. Here the outer layer of thin albumen is also quite conspicuous. The chalazae towards the narrower end of the egg is larger than that in the other. The normal twisting of the chalazae is still not very distinct.

Discussion:

According to Conrad and Phillips (1938) the layer of dense albumen next to the yolk becomes more like a fluid before the egg reaches the uterus. There, due to the rotation of the albumen around the yolk, the mucin of this fluid-like gel is segregated to form the chalaziferous layer and the chalazae. On the other hand it has been clearly shown in the present study that the chalaziferous layer is to be found/-

found even in eggs removed from the anterior magnum region.

As early as 1898 Mitrophanow described the envelope of yolk as consisting of two layers - an internal or the vitelline membrane and an external or the albuminous layer. The presence of blood spots in between the two layers led Mitrophanow to suggest that the external albuminous layer (chalaziferous layer) was secreted in the oviduct.

Lécaillon (1910,a and b) thought that the external layer was completed before the egg reached the oviduct but later on (1910c) he modified his view in favour of that of Mitrophanow (1898).

Surface (1912), Bradley (1928), Richardson (1935) and Scott and Huang (1941) have all presented evidence that the chalaziferous layer is secreted in the posterior portion of the infundibulum to which the name "chalaziferous region" of the oviduct was given by Richardson (1935). It has not been possible to confirm their observations directly as eggs from the "chalaziferous region" were not obtained. However, from

from the present observations and in the light of previous work it can be concluded that this layer is secreted in the posterior portion of the infundibulum, the true "chalaziferous region."

It would not be out of place to mention here the work of Burmester and Card (1939). They removed sections, 2-7 cm. long, from the "chalaziferous region" of the oviduct and reported that such resection was conducive to the formation of dwarf or yolkless eggs. It could be suggested that as no chalaziferous layer was secreted, due to the absence of this particular region, the delicate vitelline membrane could not remain intact while passing through the narrow oviduct. The yolk, thus set free, was either pushed back into the body cavity perhaps by antiperistalsis or was included in the dwarf eggs.

As already pointed out by Burmester and Card (1939) that the resection of the "chalaziferous region" did not have any significant effect in decreasing the weight of the chalazae, Scott and Huang (1941) also showed that the quantity of mucin secreted in that region could not possibly form the chalazae. These are, according to them, formed by the mucin secreted in/-

in the magnum region. These observations have been confirmed in the present study. Figures 3 and 4 show clearly that the mucin fibres secreted in the magnum region are added on gradually to the chalaziferous layer at the two poles of the yolk and these constitute the material for the chalazae. The normal twisting of the chalazae (Almquist, 1936) is seen only after the egg has been in the uterus for some time. This results from the rotation of the thick albumen around the yolk as already proved experimentally by Conrad and Phillips (1938). It may also be pointed out here that in the miniature eggs where, due to the absence of the normal yolk, the thick albumen does not find anything to rotate around, the normal chalazae are absent.

It is now a well established fact that the inner layer of thin albumen is present long before the egg reaches the uterus. Almquist (1936) suggests that it probably results from syneresis of a part of the dense albumen as dilution of this with water or a watery solution cannot account for the formation of this layer. To support his view he also quotes Almquist and Lorenz (1935) and Romanoff (1929), according to whom, the inner layer of thin albumen has a/-

a higher solids content than that of the dense albumen or outer layer of thin albumen. The chalaziferous layer, according to them, has even a still higher solids content.

Conrad and Phillips (1938) state that the dense albumen just near the yolk becomes more like a fluid before the egg reaches the uterus. This results not from the chemical destruction of mucin but by the breaking down of the gel structure. They could not, however, decide whether this change was brought about by some mechanical action of the oviduct or by the presence of chemical agents.

Scott and Huang (1941) noted that the staining reaction of the layer nearest the yolk changed gradually during egg formation. They, therefore, ascribed this change (from the gel to the suspension of mucin fibres) to the presence of some chemical agent.

It is also quite possible that the inner layer of thin albumen may be secreted as such by the anterior magnum region of the oviduct as would appear from the work of Asmundson and Burmester (1936). The resection of 7, 8 or 10 cm. from the anterior portion of/-

of the albumen tube resulted in the reduction of liquid albumen while the amount and percentage of firm albumen did not show any significant change. The work of Cole (1937 and 1938) also points towards the same conclusion. He suggests that the number and distribution of the mucin fibres determine the condition of a particular sample of albumen. He further states that the height of the goblet cells which secrete the mucin fibres averages 11.4 μ in the anterior portion of the magnum, 15.7 μ in the middle region, 30 μ in the posterior region and reaches a maximum of 33.9 μ at the junction with the isthmus.

There is no doubt that the outer layer of thin albumen appears only after the shell membranes have been formed, but the method of its appearance has been the subject of much discussion.

According to Pearl and Curtis (1912), the egg, during its stay in the isthmus and the uterus, receives its outer layer of thin albumen by a process of osmosis through the shell membranes. McNally (1934), by a study of the amount of various proteins in mature and immature eggs found that the former contained more of ovoglobulin than the latter. This, according to him/-

him, is taken in through the shell membranes.

Hansen (1933) doubted the observation of Pearl and Curtis (1912) as, according to him, the shell membranes must be impermeable to albumen. This doubt originated from the non-diffusion of albumen when a shell-less egg was placed in water. He, therefore, concludes that only the watery solution of salts is taken in after the shell membranes have been formed. Indirect evidence presented by Asmundson and Jervis (1933) and Asmundson and Burmester (1936 and 1938) is also in support of Hansen's view that no appreciable amount of protein enters the egg after the formation of the shell membranes.

Hughes and Scott (1936) found that after an egg was laid the greatest increase in ovoglobulin was in the inner layer of thin albumen while, according to McNally (1934) it should be in the outer layer of thin albumen. They suggest that the increase in the amount of ovoglobulin is due to a change in the solubility of the egg white proteins.

Similarly Scott, Hughes and Warren (1937) have presented evidence to show that proteins would not pass/-

pass from the uterine secretion of low protein concentration into the egg white of high protein concentration through the shell membranes.

Beadle, Conrad and Scott (1938) have found that the uterine secretion is not an albuminous solution like that which forms the outer layer of thin albumen in a laid egg. The uterine secretion, according to them, is a mineral solution made up of sodium, calcium and potassium in the form of chlorides and bicarbonates. From an analysis of uterine and laid eggs they have concluded that the principal additions to the albumen of an egg while it is in the uterus, are water, potassium and bicarbonate ions with smaller amounts of sodium and chloride ions.

That water is added to the egg while it passes through the isthmus and the anterior uterus has also been conclusively proved by Burmester (1940).

It can, therefore, be safely concluded as has already been suggested by Conrad and Scott (1938), that the outer layer of thin albumen is formed by the inflow of uterine fluid. As the uterine secretion diffuses into the egg the soluble proteins of the dense albumen come out and increase the solids content

of/-

of the outer liquid layer.

Summary.

1. The chalaziferous layer is secreted in the posterior portion of the infundibulum, the so-called "chalaziferous region" of the oviduct.

2. In eggs removed from the posterior magnum a faint whitish cloud of mucin fibres can often be observed in that end of the egg which is towards the isthmus. These mucin fibres are the precursors of the chalaza.

3. Simultaneously with the appearance of the mucin fibres the inner layer of thin albumen also becomes visible.

4. In the isthmian eggs faint whitish clouds of mucin fibres can be seen at both poles of the yolk.

5. The normal twisting of the chalazae is observed only after the egg has been in the uterus for some time. This takes place due to the rotation of the thick albumen round the yolk.

6. The outer layer of thin albumen becomes distinct only in the uterus.

7/-

7. The mode of formation of the inner and the outer layer of thin albumen is discussed.

Explanation of Plate

Fig. 1. Section of a yolk from the anterior magnum region of the oviduct. X 500.

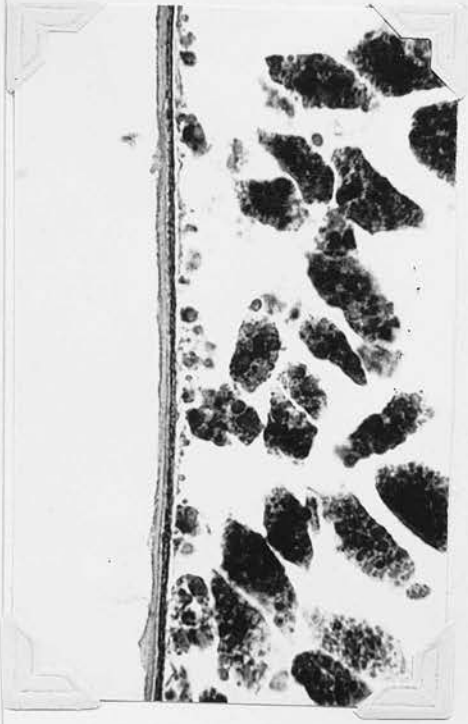
Fig. 2. Section of a yolk from the mid-magnum region of the oviduct. X 500.

Fig. 3. Section of that pole of the yolk which is towards the isthmus from an egg obtained from the post-magnum region of the oviduct.

Fig. 4. Section of that pole of the yolk which is towards the isthmus from an egg obtained from the magnum-isthmus junction.

Fig. 5. Contents of a shelled egg from the uterus.

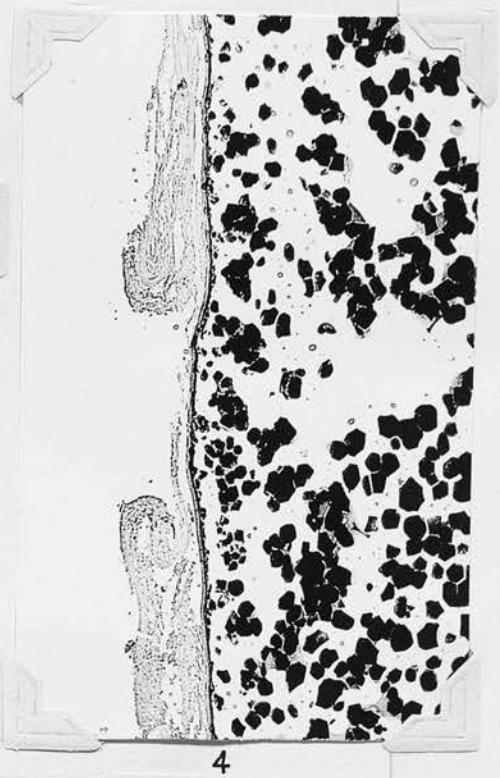
Plate



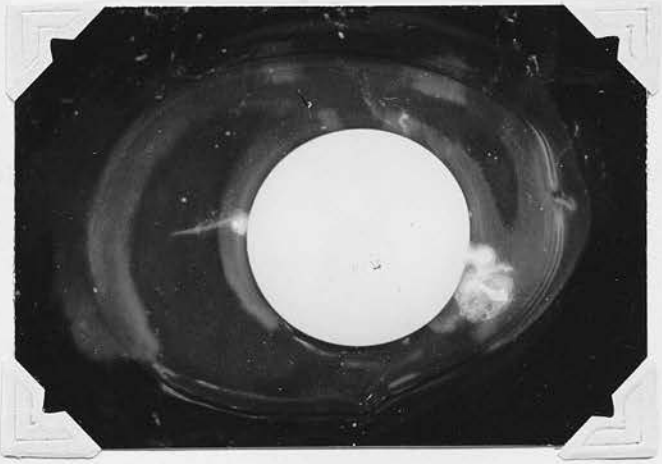
1

2

3



4



5

Part IV.

Note on An Abnormal Intrafollicular Ovum.

Hutt (1939), describing an intrafollicular ovum laid by a White Leghorn pullet, stated that it consisted of an unruptured ovarian follicle containing an apparently fully developed ovum. Another remarkable point about it was that it came down the oviduct without acquiring the usual egg envelopes namely the albumen, shell membranes and the shell. Though references to various types of abnormal eggs can be found in literature from time to time, the abnormality described by Hutt (1939) had not been reported previously according to him.

A somewhat similar egg, but with all its egg envelopes was laid at the Institute of Animal Genetics by a Brown Leghorn hen (M 456) on the 15th May, 1946. This bird was hatched on the 1st April, 1940. She laid her first egg on the 1st October the same year when she was 183 days old. The record of her egg production has been quite normal throughout. She laid 215 eggs in her first laying year, 188 in the second, 175 in the third, 152 in the fourth, 134 in the fifth and 89 in the sixth/-

sixth laying year. She is still alive and has just started laying for her seventh year. From March 3rd to March 22nd 1947 she has laid 11 eggs. Out of all the 964 eggs laid by her so far only two eggs can be classed as abnormal. The first of these was laid on the 12th March 1942 when the bird was in her second laying year and it was abnormal inasmuch as it contained a blood spotted yolk.

The second abnormal egg which forms the subject of this paper was laid by this bird in her sixth laying year during which she produced 89 eggs in all. The weight of these eggs varied from 57 to 67 grammes but the abnormal egg weighed only 46 grammes and it was, therefore, classed as miniature. Though no egg was laid on the preceding and two succeeding days yet it was nothing unusual for the bird to exhibit such periods of rest. From its shape and outward appearance this egg appeared to be quite normal (Plate I, fig. 1).

When first examined the shell and the outer shell membrane at the broad end of the egg had already broken and the thickened and dried up tip of a blood streak was in view in the air chamber. On opening the/-

the egg the blood streak, which was covered by a whitish membrane, was seen penetrating the egg albumen and ending in a solid greyish white mass. Towards the outside the whitish membrane covering the blood streak seemed to be continuous with the inner shell membrane. The albumen was suffused with blood and no definite chalazae could be made out. A careful examination of the greyish white central mass revealed it to be the complete follicle extensively supplied with blood vessels. However, when opened the follicle was seen to contain not a normal yolk but a liver-like mass. A few pieces of this substance were fixed in Bouin's and sections prepared for histological study with the usual Delafield's haematoxylin and Eosine technique. An extensive study of these sections revealed the liver-like mass to be mainly made up of red blood corpuscles. In some sections, however, the mass of blood cells was seen to be covered by a membrane (possibly the vitelline membrane) as yolk granules though mixed with blood cells could be seen on the other side of it (Plate I, fig. 2). The presence of cells like those of the follicular epithelium lining the membrane at some places towards the main mass of blood cells also points

to/-

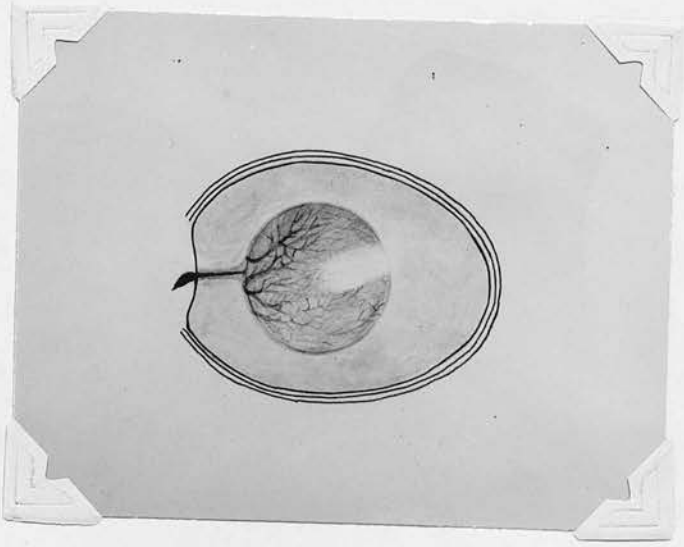
to the same conclusion that the membrane in question is the vitelline membrane. It, therefore, appears that due to some unknown cause copious intrafollicular bleeding took place in the follicle at a very early stage when it was not even ready for ovulation. This bleeding resulted in the formation of a blood streak and also the central liver-like mass. The whitish membrane covering the blood streak was identified as the pedicel of the follicle.

Explanation of Plate

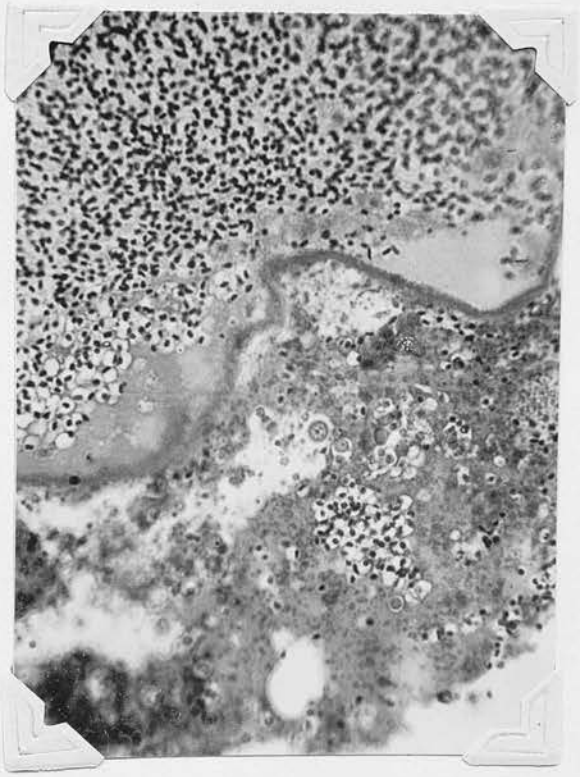
Fig. 1. (diagrammatic) The contents of the abnormal egg. (Approximately natural size).

Fig. 2. Section of a piece of the liver-like mass contained inside the follicle. X 350.

Plate I



1



2

Acknowledgments

It is a pleasure to express my sincere gratitude to Dr A. W. Greenwood under whose supervision this work has been carried out. I am grateful to him not only for his valuable suggestions and for the provision of laboratory facilities but also for placing at my disposal all his personal records. To Dr J. S. S. Blyth I am deeply indebted for her extreme kindness and ever-ready help in the preparation of the manuscript. My thanks are also due to Mr G. R. Knight for taking the photomicrographs and for his technical assistance.

Literature Cited.

1. Adelmann, H. B., 1942. The embryological treatise of Hieronymous Fabricius of Aquapendente. Cornell Univ. Press, Ithaca, New York (Quoted by Nalbandov and Card, 1944).

2. Almquist, H. J., 1936. Formation of the Chalazae in the hen's egg. Poul. Sci., 15: 460-461.

3. _____ and Lorenz, F. W., 1933. The Solids content of egg white. Poul. Sci., 12: 83-89.

4. Aristotle. Transl. by A. Platt, 1910. Degeneratione animalium III, 2, 752. C 1-10 (Quoted by Nalbandov and Card, 1944).

5. Asmundson, V. S., 1931. The Formation of the Hen's Egg. Sci. Agri. 11, 1-50.

6. _____ and Burmester, B. R., 1936. The Secretory Activity of the Parts of Small Hen's Oviduct. J. Exp. Zool., 72: 225-246.

7. _____ 1938. The Effect of Resecting a part of the Uterus on the Formation of the Hen's Egg. Poul. Sci., 17: 126-130.

8./-

8. Asmundson, V. S. and Jervis, J. G., 1933. The Effect of Resection of Different Parts of the Oviduct on the Formation of the Hen's Egg. J. Exp. Zool., 65: 395-420.

9. Beadle, B. W., Conrad, R. M., and Scott, H. M., 1938. Composition of the Uterine Secretion of the Domestic Fowl. Poul. Sci., 17: 498-504.

10. Benjamin, E. W., and Pierce, H. C., 1937. Marketing poultry products. John Wiley and Sons, Inc., New York (Quoted by Burmester and Card, 1938).

11. Bradley, O. C., 1928. Notes on the Histology of the Oviduct of the Domestic Hen. J. Anat., 62: 339-345.

12. Burmester, B. R., 1940. A Study of the Physical and Chemical Changes of the Egg during its passage through the isthmus and uterus of the Hen's Oviduct. J. Exp. Zool., 84: 445-500.

13. _____ and Card, L. E., 1938. On the Nature of "meatspots" in Eggs. Poul. Sci., 17: 235-239.

14/-

14. Burmester, B. R., Card, L. E., 1939. The Effect of Resecting the so-called "Chalaziferous Region" of the Hen's Oviduct on the Formation of subsequent Eggs. Poul. Sci., 18: 138-145.

15. Card, L. E., and Nalbandov, A., 1944. Controlling Blood and Meat Spots (Abs.) Poul. Sci., 23: 551.

16. Cole, R. K., 1937, Histological Basis for Differences in Consistency of Firm Egg Albumen. Poul. Sci., 16: 356.

17. _____ 1938. Histology of the Oviduct of the Fowl in Relation to Variations in the Condition of the Firm Egg Albumen. Anat. Rec., 71: 349-361.

18. Conrad, R. M. and Phillips, R. E., 1938. The Formation of the Chalazae and Inner Thin White in the Hen's Egg. Poul. Sci., 17: 143-146.

19. _____ and Scott, H. M., 1938. The Formation of the Egg of the Domestic Fowl. Physiol. Rev., 18: 481-494.

20/-

20. Crew, F. A. E., 1930. Eggs of Abnormal Size, Shape and Consistency. The Feathered World.

21. Denton, C. A., 1947. Observations on the Incidence and Characteristics of Blood and Meat Spots in Hen's Eggs. Poul. Sci., 26: 272-276.

22. Féré, Ch., 1898. Note sur le poide de l'oeuf de poule et sur ses variations dans les pontes successives. Jour. de l'Anat. et de la Physio., 34: 123-127.

23. Halnan, E. T., and Day, H. D., 1935. An analysis of some egg faults. J. Min. of Agr., 42: Part I - 236, Part II - 326.

24. Hansen, C. H., 1933. On the formation of the Hen's Egg with especial Reference to the White. Proc. Fifth World's Poul. Congr. 2: 587-593.

25. Hughes, J. S., and Scott, H. M., 1936. The Change in the Concentration of Ovoglobulin in Egg White during Egg Formation. Poul. Sci., 15: 349-351.

26. /-

26. Hutt, F.B., 1939. An intrafollicular ovum laid by a fowl. Poul. Sci., 18: 276-278.

27. Jeffrey, F.P., 1945. Blood and meat spots in chicken eggs. Poul. Sci., 24: 363-374.

28. _____ and Pino, J., 1943. The effects of heredity and of certain environmental factors on the incidence of blood spots in chicken eggs. Poul. Sci., 22: 230-234.

29. Lécaillon, A., 1910a. Sur la structure et la signification de la membrane qui enveloppe la sphère vitelline de l'oeuf des Oiseaux. Compt. Rend. Acad. Sci., 150. 240-242.

30. _____ 1910b. Nouvelles observations sur la capsule vitelline de l'oeuf du Merle commun (Turdus merula L.) Compt. Red. Soc. Biol., 68: 218-219.

31. _____ 1910c. Troisième note relative a la structure et a la signification de la capsule vitelline de l'oeuf du Merle commun. Compt. Red. Soc. Biol., 68: 284-286.

32. /-

32. Lerner, I. M., 1946. The incidence of blood spots in eggs and its relation to first year mortality. Poul. Sci., 25: 392-394.

33. _____ and Smith, W. R., 1942. Effect of season and heredity on the incidence of blood spots (Abs.) Poul. Sci., 21: 473.

34. Lewis, H. R., 1913. Productive Poultry Husbandry Philadelphia, London. (Quoted by Pearl and Curtis, 1916).

35. Lucas, A. M., 1946. Hematology of blood spots in eggs of White Leghorn Chickens. Amer. Jour. Anat., 79: 431-471.

36. McNally, E., 1934. Passage of ovoglobulins through the shell membrane. Proc. Soc. Exp. Biol. and Med., 31: 946-947.

37. Mitrophanow, P., 1898. Note sur la structure et la formation du l'enveloppe du Jaune d'oeuf de la poule. Bibliographie Anatomique, 6: 69-84.

38. Nalbandov, A. V., and Card, L. E., 1941. Further evidence on the cause and occurrence of blood and meat spots (Abs.) Poul. Sci. 20; 469.

39/-

39. Nalbandov, A. V., and Card, L. E., 1944. The problem of blood clots and meat spots in chicken eggs. Poul. Sci., 23: 170-180.

40. Pearl, R., and Curtis, M. R., 1912. Studies on the physiology of reproduction in the domestic fowl. V. Data regarding the physiology of the oviduct. J. Exp. Zool. 12: 99-132.

41. _____ 1916. Studies on the physiology of reproduction in the domestic fowl. XV. Dwarf eggs. Jour. Agr. Res. 6: 977-1042.

42. _____ 1916. Dwarf eggs of the domestic fowl. Bull. 255 from the Annual Report of the Maine Agric. Exp. Station, 289-328.

43. Pearl, R., Surface, F. M., and Curtis, M. R., 1911. Poultry Diseases and their treatment. Orono, 1-216 (Quoted by Pearl and Curtis, 1916).

44. Quinn, J. P., and Godfrey, A. B., 1940. Inheritance and variation of blood spots in chicken eggs (Abs.). Poul. Sci., 19: 359.

45/-

45. Richardson, K. C., 1935. The secretory phenomena in the oviduct of the fowl including the process of shell formation examined by the microincineration technique. Phil. Trans. Roy. Soc. Lond., B. 225: 149-195.

46. Romanoff, A. L., 1929. The dry matter in different layers of egg albumen. Science, 70: 514.

47. Scott, H. M., and Huang, W. L., 1941. Histological observations on the formation of the chalaza in the hen's egg. Poul. Sci., 20: 402-405.

48. _____, Hughes, J. S., and Warren, D. C., 1937. Augmentation of nitrogen to the egg white after the formation of the shell membranes in the fowl. Poul. Sci., 16: 53-61.

49. Surface, F. M., 1912. The histology of the oviduct of the domestic hen. Maine Agr. Exp. Sta. Bull., 206: 395-430.

50. Van Wagenen, A., Hall, G. O., and Wilgus, H. S., 1937. Variations in egg quality characters of certain breeds, varieties and strains of chickens. J. Agr. Res. 54: 767-777.

51. Warner, D. E., and Kirkpatrick, W. F., 1916. What the size of an egg means. Jour. Heredity, 7: 128-131.